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Immunologic Mechanisms of Parenchymal Lung Injury

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The lung, like most other organs, is susceptible to injury by circulating immune complexes, and also by humoral autoantibody and immune lymphocytes which specifically recognize selected lung antigens. In addition, by virtue of its direct communication with the external environment, the lung can also be injured by inhaled environmental agents which trigger inflammatory reactions mediated by immune effector systems. Although major emphasis to date has been placed on the ability of inhaled antigens to first sensitize, then provoke, immunologically specific reactions in the lung, there is increasing evidence to show that these same immune effector systems are also triggered in an immunologically nonspecific fashion by a certain environmental agents (termed "mitogens") which activate leukocytes in a polyclonal fashion. Such agents include certain viruses and other microorganisms, bacterial endotoxin, a wide variety of plant lectins, and certain chemicals, such as the phorbol esters. Although such agents act in an immunologically nonspecific fashion, they are nonetheless quite specific from a chemical viewpoint, and in many cases act by binding to specific receptors on the cell surface. By activating macrophages directly, and by activating much larger percentages of a given lymphocyte population than do specific antigens, they induce correspondingly amplified inflammatory reactions *in vivo*. Recent studies with animal models indicate that inhaled mitogens are strikingly effective in inducing pulmonary inflammation, whereas inhaled antigens (lacking mitogenic activity) produce little if any parenchymal injury in immunized recipients, unless administered in conjunction with a mitogen. Ongoing studies using such models promise to provide valuable new insight into the biologic properties which govern the pathogenicity of inhaled environmental agents, the mediators they release, and the biochemical basis for variations in individual susceptibility to injury by such agents.

Introduction

The association between inhalation of organic dusts and the development of chronic pulmonary disease has been recognized for centuries (*1*). However, whereas the actions of such dusts have traditionally been considered to be a nonspecific "toxic" effect, advances in our understanding of tissue inflammation have led to revised, more detailed hypotheses concerning mechanisms of tissue injury. Studies of environmental lung disease have been particularly influenced by related advancements in pulmonary immunology and immunopathology, perhaps best exemplified by the delineation of IgE-mediated allergic asthma (an airway disorder which will not be discussed in this paper), and the production of pulmonary hemorrhage and acute glomerulonephritis by antibasement membrane antibodies in patients with Goodpasture's syndrome (*2*).

Currently, there is considerable interest in immunologic mechanisms which may be responsible for environmental lung diseases associated with dust inhalation. Such studies were prompted largely by Pepys (*3*), who, nearly two decades ago, demonstrated that sera from patients with farmer's lung contained precipitating antibody against the causative agent, *Micropolyspora faeni*. Subsequently, a number of environmental lung diseases, characterized by the presence of precipitating serum antibody against pathogenic dust antigens, have been described (*4*). Pepys proposed that these pulmonary diseases, which he termed "extrinsic allergic alveolitis" have also been known as "hypersensitivity pneumonitis," result from local formation of pathogenic immune complexes composed of inhaled antigen and humoral antibody.

There are serious objections to such a straightforward immune complex hypothesis, however, as Pepys himself noted (*5*). First, it has been well established that many, if not most, individuals who inhale these pathogenic dusts develop humoral antibodies to dust antigens but fail to develop evidence of disease (*6*). Second, there is little if any obvious relationship between the magnitude or specificity of individual immune responses to dust antigens and the extent of pulmonary disease (*7*). This is in contrast to the cutaneous Arthus reaction and other immune complex-mediated phenomena. Third, whereas virtually all dust antigens, when inhaled, stimulate production of specific host antibody (*8*, *9*), only selected antigens (e.g., pigeon droppings) appear to be pathogenic; other equally antigenic, osten-

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sibly similar antigens (e.g., chicken droppings) seldom if ever cause allergic alveolitis (*5*). Pepys (*5*) has thus further proposed that immune complex injury occurs in association with a more rapidly developing, immediate-type hypersensitivity response which potentiates subsequent immune complex formation. Interestingly, however, Pepys (*10*) has noted that patients with allergic alveolitis are notably nonatopic, and that these immediate-type hypersensitivity reactions are not mediated by IgE-class antibody.

Subsequently, several alternative hypotheses have been proposed which, in essence, identify both cellular and humoral mechanisms as the prime cause of lung injury (*7*); such hypotheses have been based largely on demonstrations of cellular as well as humoral immunity to dust antigens in patients with allergic alveolitis. As with Pepys' original hypothesis, however, they fail to account for the differential pathogenicity of various dust antigens, and also the poor correlation between test measurements of host immunity and the severity of lung disease. In fact, many now believe that host immunity to environmental dust antigens merely reflects a history of exposure.

Clinical data also suggest that noninfectious parenchymal lung diseases other than allergic alveolitis may also be immunologically mediated; both fibrosing alveolitis (*11*) and Wegener's granulomatosis (*12*), for example, have been ascribed to immune complex-type reactions. Moreover, immune complexes have been identified in the circulation of a variety of patients with active interstitial pneumonitis (but not end-stage fibrosis), including patients with "idiopathic" interstitial inflammation, as well as in patients with other known immune complex-mediated connective tissue disorders (*13*).

Yet a new dimension has been recently added to our understanding of "immunologic" disorders, namely, the recognition that both the cellular and humoral effector systems can also be triggered through immunologically nonspecific mechanisms, as, for example, the acute respiratory distress syndrome caused by nonimmune activation of complement in patients undergoing extracorporeal dialysis (*14*). This paper will not attempt to outline the entire spectrum of human lung diseases thought to be immunologically mediated, nor will it review in detail all known immune effector systems, but rather it will concentrate on recent advances in our understanding of how immune effector systems might be activated in the lung. In particular, we will highlight the potential role of environmental and endogenous cell activators, emphasizing the pathogenic consequences associated with the release of certain inflammatory mediators by activated lung cells.

Immune Effector Systems

Immunologic tissue injury is mediated through activation of one or more effector systems. Immunologic specificity exists only during the recognition and binding of antigen to antigen-specific lymphocyte receptors or immunoglobulin combining sites. Such initial reactions are not of great pathological moment in themselves, but serve to trigger cellular and humoral effector systems (such as macrophages and complement, respectively). These systems, through a process of self-activation and self-amplification, generate soluble inflammatory mediators, which recruit and activate additional leukocytes. When fully developed, such a process constitutes a true inflammatory nidus, recognized clinically and histologically.

Although the activation of the immune effector systems in such instances is immunologically specific in nature, the effector systems themselves function in an immunologically nonspecific fashion. These effector/amplification systems are, moreover, controlled and regulated by discrete inhibitor systems, including beta-adrenergic agonists, E-series prostaglandins and histamine (*15*), and in the lung, surface-active materials as well (*16*). Depending on the site and manner in which effector systems are activated (and regulated), they may serve either to protect the host or to cause tissue injury and disease.

Immune effector systems comprise both humoral and cellular elements, and attempts to characterize immunologic disorders as being one or the other in nature constitute an artificial distinction. For example, effector mechanisms associated with "cellular" reactions (such as the tuberculin skin reaction) are mediated in part by soluble factors; indeed, certain "humoral" factors, such as complement, are produced by activated monocytes and macrophages (*17*). Traditionally, humoral immune effector mechanisms have included the complement, kinin and coagulation systems. The latter two systems will not be considered further, and the reader is referred to several excellent reviews which discuss these areas in depth (*18,19*).

Complement

Activation of the complement system is characterized by the sequential proteolytic cleavage of complement protein components, thereby converting inactive precursor forms into biologically active fragments (Fig. 1). While the complement components are typically found in the plasma, they can also be synthesized locally by macrophages (*17*), indicating that complement-mediated reactions could theoretically occur in the lung air spaces in the absence of other humoral constituents. However, studies by Reynolds et al. (*20*) argue against such a possibility by showing that ratios of complement components to serum albumin, as measured in human bronchopulmonary lavage fluid, are lower than those in serum. Among the biologic activities associated with these fragments are: increases in vascular permeability (C3a; C5a), contraction of smooth muscle (C3a; C5a), enhanced phagocytosis (C3b;), leukocyte chemotaxis (C5a) and cell lysis (C5b-9). These biologic activities are in turn controlled not only through spontaneous decay, where biologic activity decreases spontaneously with

time, but also through the action of serum inhibitors, such as the C1-esterase inhibitor, C3b inactivator and β_1H (*21, 22*). Absence of the C1-esterase inhibitor is associated with episodes of laryngeal edema (hereditary angioneurotic edema) (*23*), while the inability of the C3b inactivator and β_1H control protein to effectively regulate the formation of the alternative pathway C3-cleaving enzyme, BbC_3b (due either to a C3b inactivator deficiency, or to the binding of an autoantibody to the C3bBb complex), is associated with a hypocomplementemic form of glomerulonephritis (*24*).

The complement cascade can be activated by two major pathways. The "classical" pathway is initiated by binding and activation of the C1q,r,s assembly, leading in turn to sequential activation of C4, C2, C3 and C5-9. The "alternative" pathway bypasses the C1, C4 and C2 steps, utilizing instead factors B and D plus properdin, C3, and C5–9 (*25*). Immune complexes of antibody and antigen activate complement primarily via the classical pathway. Both pathways can be activated, however, in an immunologically nonspecific fashion by a variety of

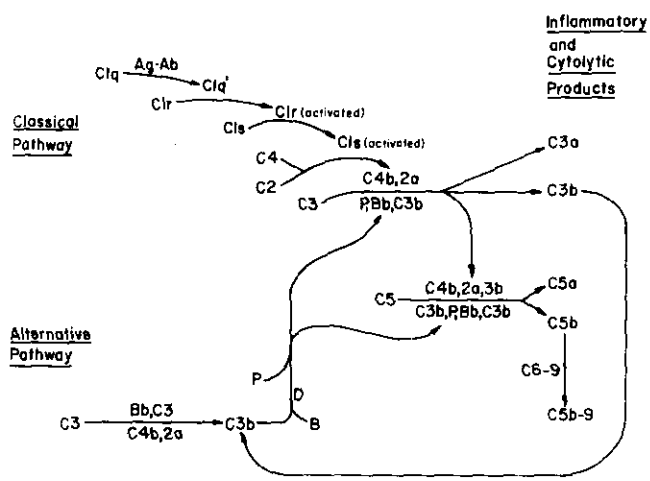


FIGURE 1. Schematic representation of the complement activation pathways. In the classical pathway, as activated by antigen–antibody complexes, Clq is altered by binding to the Fc immunoglobulin region, leading to successive activation of Clr and then Cls. Cls enzymatically cleaves C4 and C2 to generate the C3-cleaving enzyme, C4b,2a, which combines with C3b to form the C5-cleaving enzyme, C4b,2a,3b. In the alternative pathway, assembly of the C3-cleaving enzyme, P,Bb,C3b, begins with binding of nascent C3b to a suitable activating surface (such as bacterial polysaccharides), followed by binding of Factor B, which is then activated to Bb through enzymatic cleavage by Factor D. This C3-cleaving complex, Bb,C3b, is stabilized by binding of properdin (P) to a site on C3b. A C5-cleaving enzyme is then formed by the uptake of a second C3b to yield C3b,P,Bb,C3b. The small amount of nascent C3b required to initiate this autocatalytic process is slowly generated by the inefficient enzyme, Bb,C3, normally present in blood, (or, alternatively, by C4b,2a from the classical pathway). Two control proteins, β_1H and C3b inactivator, however, effectively inhibit the subsequent assembly of P,Bb,C3b in the absence of an alternative pathway activator. The C3- and C5-cleaving enzymes generated through either pathway, produce the inflammatory fragments, C3a, C3b and C5a, and also lead to the generation of the membrane attack assembly, C5b-9. From Mayer (*21*) with permission.

polymeric agents. For example, microbial polysaccharides can activate the alternative pathway, while saltlike complexes of DNA and lysozyme activate via the classical pathway (*26*). Of particular interest, lipopolysaccharide (LPS) activates complement (via the alternative pathway), and also B-lymphocytes and macrophages, in an immunologically nonspecific fashion (see below).

Another important aspect of the complement system is that it is activated via an enzymatic cascade, with proteolytic cleavage of precursor components yielding enzymatically active fragments which, in turn, cleave (and "activate") succeeding components. By virtue of its enzymatic nature, the overall reaction is, in effect, amplified. In particular, generation of the intermediate, C3b, constitutes a major amplifying step in the alternative pathway by acting through a positive feedback loop (see Fig. 1).

Lymphokines and Monokines

A number of cell-derived mediators have also been characterized *in vitro*. In some cases, these mediators exist as preformed substances within the cell (e.g., histamine within mast cell granules) and are released into the extracellular environment following an appropriate stimulus. In other cases, stimulation of the cell leads first to mediator synthesis, then release (e.g., lymphokine production by lymphocytes). The number of biologic activities generated by activated cells is very large; a partial list is given in Table 1. The lymphokines, which at first were thought to be released exclusively by T-(thymus dependent) lymphocytes, are now known to be produced, in some cases, by B-(bone marrow-derived) lymphocytes as well (e.g., migration inhibitory factor) (*27*). Only some of these mediators have been characterized physicochemically as yet, and it is possible that, in some instances, different biologic activities may in fact represent different manifestations of the same molecule.

Soluble mediators released by activated lymphoid cells include Interleukin 1 (IL-1; also known as "lymphocyte activating factor"), Interleukin 2 (IL-2; also known as "T-cell growth factor"), "mitogenic factor," "macrophage activating factor" and chemotactic factors, which act in concert to attract, then activate other leucocytes in an immunologically nonspecific fashion. These mediators constitute an important amplifying mechanism and, as in the case of the complement cascade, enable the host to mount a sizeable inflammatory response following a relatively trivial but immunologically specific initiating reaction. In classic studies by McCluskey et al. (*28*) and later by Cohen et al. (*29*), immune cell participation in delayed-type hypersensitivity reactions was examined in recipients passively sensitized with radiolabeled lymphocytes. These studies demonstrated that virtually all lymphocytes present at the inflammatory site were in fact "nonimmune" (i.e., they were of host origin). Presumably, such cells were recruited (and activated) by amplifying mediators.

Table 1. Representative cell-derived mediators.

Mediator	Mediator class	Cell source	Biological action(s)
Interleukin 2, mitogenic factor, T-cell replacing factor	Lymphokines	Lymphocyte	Induce lymphocyte growth/proliferation and/or maturation
Chemotactic factors	*Lymphokines*	*Lymphocyte*	*Individual factors chemotactic for neutrophils, monocytes, eosinophils or basophils*
Vascular permeability factor	Lymphokine	Lymphocyte	Increases vascular permeability
Lymphotoxin	Lymphokine	Lymphocyte	Cytotoxic for L-cells and selected tumor cells
Migration inhibitory factor	Lymphokine	Lymphocyte	Immobilizes macrophages
Macrophage activating factor	Lymphokine	Lymphocyte	Activates macrophages
Fibroblast activating factor	Lymphokine/ *monokine*	Leukocyte	Stimulates proliferation/activation of fibroblasts
Interleukin 1 (lymphocyte activating factor)	Monokine	Macrophages/ monocytes	Activates lymphocytes; stimulates IL-2 production; induces fibroblast proliferation; causes fever
C3a,C5a	Complement	Macrophages/ monocytes	Causes cellular release of histamine; C5a also chemotactic for neutrophils
Histamine, serotonin	Vasoactive amines	Basophils, mast cells, platelets	Increase vascular permeability; contract smooth muscle
Platelet activating factor (AGEPC)	*Phospholipid* derivative	*Macrophages*, basophils, neutrophils	*Induces platelet aggregation and secretion of vasoactive amines*; also stimulates neutrophil aggregation and secretion
PGE_2, $PGF_{2\alpha}$, thromboxane, prostacyclin	Arachidonate derivatives	Leukocytes	Diverse (and even opposing) inflammatory effects, depending on the mediator; PGE_2 also acts as an immunomodulator
5-HETE, 12-HETE	Arachidonate derivatives	Leukocytes	Chemotactic for neutrophils; induce neutrophil granule release
Leukotrienes B_4, C_4, D_4, E_4	Arachidonate derivatives	Leukocytes	LTC_4, LTD_4, LTE_4 contract smooth muscle and increase vascular permeability; LTB_4 is chemotactic for neutrophils
Superoxide anion, hydrogen peroxide	Oxygen-derived free radicals	Macrophages, neutrophils	Cytotoxic for endothelial cells, tumor cells

IL-1 is of particular interest since it provides one of the macrophage-derived signals necessary for both the induction as well as the expression of the immune response. In addition, it also is required for T-cell responses to mitogens, and its ability to greatly amplify such responses constitutes the basis for the IL-1 assay (*30*). Its role in the production of inflammatory lung disease seems certain since alveolar macrophages, when activated, produce IL-1 in large amounts, and since IL-1 also appears to be identical to endogenous pyrogen (*31*). In the case of rabbit alveolar macrophage IL-1, we have shown the production of two distinct species possessing molecular weights of 14,400 and 11,600 daltons, and isoelectric points of 4.6 and 7.4, respectively (*32*). Recently, IL-1 has also been reported to cause fibroblasts to proliferate (*33*), and synovial cells to secrete collagenase (*34*). Thus, this single mediator, produced by alveolar macrophages, helps establish immunity to antigenic substances, facilitates activation of cellular immune effector system by both antigens and mitogens, produces fever, and may lead to pulmonary fibrosis.

As in the complement system, in which component proteins exist as inactive precursor forms and become biologically active only following enzymatic cleavage, cell-derived mediator synthesis and/or release typically occurs only following activation of the cell by an appropriate stimulus. This can occur in an immunologically specific fashion, as when antigen binds to receptors on immune T-lymphocytes, or to antigen binding sites of homocytotropic antibody on the surface of basophils. It can also occur, however, as a consequence of immunologically nonspecific cell activation by a variety of agents termed "mitogens" or "polyclonal cell activators" (*35*) (see discussion of activation systems). Among the cell activators known to trigger lymphokine and monokine production by lymphocytes and macrophages are plant lectins, microorganisms and their constituents (including LPS), and certain chemicals and enzymes (see Table 2). Polyclonal cell activators can also trigger basophils to release vasoactive amines (*36*) which, in turn, promote tissue inflammation by enhancing passage of other humoral and cellular effectors out of the blood vessels and into the interstitial spaces.

Platelet-Activating Factor

IgE-coated basophils, when stimulated with specific antigen, release not only vasoactive amines directly, but also platelet-activating factor (PAF) which, in turn, causes release of additional vasoactive amines from platelets. PAF, a potent, biologically active lipid recently identified as acetyl glyceryl ether phosphorylcholine (AGEPC) (*37*), is also released by stimulated monocytes, neutrophils, mast cells and, of particular relevance to this discussion, alveolar as well as peritoneal macrophages (*38*). *In vivo* studies in experimental animal models have shown that PAF, when injected intravenously, causes depletion of circulating neutrophils, basophils and platelets, with pulmonary platelet sequestration (*39*), increases vascular permeability, promotes immune complex deposition in serum sickness (*40*) and causes bronchoconstriction (*41*). It has been further suggested that variations in PAF release from alveolar macrophages reflect different states of activation of these cells (*38*).

Table 2. Polyclonal cell activators.

Plant lectins
Phytohemagglutinin (PHA)
Concanavalin A (Con A)
Pokeweed mitogen (PWM)
Peanut agglutinin
Soybean agglutinin
Walnut extracts
Corn extracts
Microbial organisms and their products
Gram negative endotoxin (LPS)
Staphylococcal A protein; enterotoxin B
Streptolysin S
Peptidoglycans (*E. coli; B. megaterium*)
Pneumococcal polysaccharide (S_{III})
Micropolyspora faeni
Herpes simplex
Epstein-Barr virus
Nocardia sp.
Endogenous cell-derived mediators
Mitogenic factor
Interleukin 1 (lymphocyte-activating factor)
Interleukin 2 (T-cell growth factor)
Macrophage-activating factor
Miscellaneous mitogens
Trypsin; papain
Neuraminidase/galactose oxidase
Sodium periodate
Phorbol esters
High molecular weight polymers (dextran sulfate; poly I-C; keyhole limpet hemocyanin)
Calcium ionophores (A-23187)
Mercury; zinc; nickel
Anti-immunoglobulin antibodies

Arachidonic Acid Metabolites

A fourth major immune effector system consists of a family of lipidlike mediators which are generated through metabolism of arachidonic acid, derived from membrane phospholipids by the actions of phospholipases (*42*). These mediators, in turn, can be subdivided into products of the cyclooxygenase pathway (including prostaglandins, prostacyclin and thromboxanes), and those produced through the lipoxygenase pathway (leukotrienes, including B_4 and SRS-A; HPETEs and HETEs) (Fig 2). Both classes of oxygenation products are known to be synthesized in the lung, primarily by alveolar macrophages (*43*).

The prostaglandins have been studied extensively *in vitro* and *in vivo*, often by blocking their production by aspirin and indomethacin, which inhibit cyclooxygenase. Potentially, they can function both as effectors and regulators of inflammation *in vivo*, but their precise role in tissue inflammation is somewhat difficult to define since different products may have opposing biologic activities. Moreover, they may be produced simultaneously, but in different quantities, and may have different biologic half-lives. Nevertheless, the well-known anti-inflammatory actions of aspirin almost certainly results from inhibition of cyclooxygenase activity *in vivo*. Interestingly, the lung not only is an important site for prostaglandin synthesis, it also is a major site for inactivation of certain prostaglandins (*44*).

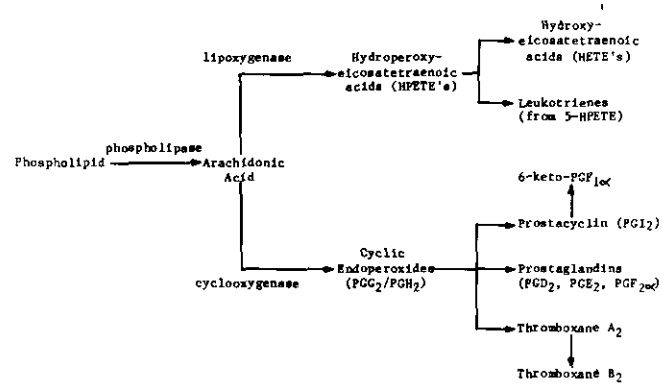


FIGURE 2. Schematic representation of the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism.

Arachidonic oxygenation products of the lipoxygenase pathway, on the other hand, are relatively insensitive to the action of the nonsteroidal anti-inflammatory drugs. SRS-A ("slow-reacting substance of anaphylaxis"), which is known to play a role in anaphylaxis, has been shown to be leukotrienes C_4 and D_4 (*45*). The chemotactic and degranulating properties of certain HETE molecules (*46*) as well as leukotriene B_4 suggest that they, too, may play a role in immunologic lung disease.

Oxygen-Derived Free Radicals

Last, a fifth effector system has recently emerged which may be important in parenchymal lung disease, and which involves the extracellular release of oxygen-derived free radicals, particularly H_2O_2, $OH^{\cdot}$ and O_2^-, by stimulated neutrophils and macrophages. These radicals are known to be important mediators of both the intracellular killing of phagocytized microorganisms and, in some cases, the extracellular killing of tumor cells by macrophages (*47*). They have been implicated in both the direct cutaneous Arthus reaction (*48*) as well as a reverse passive Arthus reaction produced in the lung (*49*), since inflammation associated with these reactions can be blocked by introduction of enzymatic "scavengers" capable of rapidly inactivating oxygen free radicals. *In vitro* studies by Jacob and co-workers (*50*) have demonstrated that biologically active complement fragments can cause neutrophils to release oxygen radicals into the extracellular environment, and that these radicals damage endothelial cells growing in culture. They have proposed that such reactions may be responsible for the acute respiratory distress syndrome in man. Unfortunately, attempts to reproduce this disorder in an animal model have been unsuccessful (*51*).

Activation Systems Which Initiate Parenchymal Lung Disease

The preceding section describes known effector systems capable of mediating immunologic tissue injury. It

should be emphasized, however, that these systems exist in normal as well as diseased individuals, and are, in fact, primarily protective for the host (*52*). Whether a particular system acts to benefit the host or, instead, cause disease is determined by the conditions of activation, especially the nature of the initiating signal, as well as by the effectiveness of relevant control or regulating mechanisms in modulating and, eventually, terminating the effector system once activated.

Immunologically Specific Mechanisms

The "classical" means by which immune effector systems are activated is through interaction of antigen with specific antibody or sensitized lymphocytes. In the case of immunologically mediated diseases, these interactions can be further subdivided into (a) reactions directed against antigens which are immunologically unrelated to the target organ affected, and (b) reactions directed against one or more antigens intrinsic to the organ structure. Examples of the first case include lung injury associated with circulating antigen–antibody complexes arriving via the circulation which become localized or "trapped" in the lung, or by complexes forming *in situ* between inhaled antigens and circulating (or locally synthesized) antibody. An example of the second case is the formation of autoantibodies, specific for capillary basement membrane antigens, and which, when they bind *in vivo*, cause pulmonary hemorrhage and glomerulonephritis (Goodpasture's syndrome). While sometimes described as an example of a "cytotoxic" mechanism (*53*), such injury in fact appears to result from damage to the extracellular basement membrane, presumably through local release of proteolytic enzymes (*54*) and, possibly, oxygen radicals.

Antigens can also activate immune lymphocytes, either by binding to cell membrane antibody (B-cells) or by interacting with immunologically specific T-cell membrane receptors. B-cell activation typically leads to B-cell proliferation and differentiation, accompanied by synthesis and release of specific antibody and certain lymphokines as well (*27*). Stimulation of T-cells results in lymphokine release, cell proliferation and differentiation. While antibody production *per se* does not necessarily result in tissue inflammation, local release of lymphokines probably does.

Immunologically Nonspecific Mechanisms

Yet another means by which immune effector systems can be activated is through the actions of mitogens, also known as "polyclonal cell activators," which activate nonimmune lymphocytes, macrophages, basophils, mast cells and complement in an immunologically nonspecific fashion (*35,55*). Best studied of these mitogens are the plant lectins, phytohemagglutinin (PHA) and concanavalin A (Con A), which bind to specific sugar moieties or "receptors" on cell surfaces. When they bind to T-lymphocytes, they further initiate a sequence of biochemical and morphological events which transform the cell from a "resting" state to an "activated" one in which the cells increase in size, synthesize new enzymes, produce and secrete lymphokines, undergo blastogenesis and cell division and, ultimately, acquire differentiated cell functions such as cytotoxic capabilities (*35*). Since these effects parallel those induced in "immune" T-cells by specific antigen, these lectins came to be widely used for *in vitro* studies of cell-mediated immunity. In this regard, they offered notable advantages over the use of antigen in that: they were effective on cells from "immunologically-naive" (i.e., not previously immunized) donors; by acting on lymphocytes in a polyclonal fashion, they activated larger numbers of lymphocytes than did specific antigen, thereby exaggerating the *in vitro* responses; and mitogens were useful in defining subsets of lymphocytes, based on their ability or failure to respond to a particular mitogen.

Subsequently, T-cell mitogens have been used to simulate cell-mediated or delayed-type hypersensitivity reaction *in vivo* as well, first in the skin, and subsequently in the lung and other organs (*56,57*). However, as yet little consideration has been given to the possible role of mitogens *per se* as a cause of environmental lung disease in man, although experimental studies have clearly demonstrated that mitogens are capable of causing a rapidly developing pneumonitis by themselves and, under certain circumstances, triggering immune complex reactions leading to pulmonary fibrosis. (*57*). Moreover, the list of known mitogens presently includes viruses, bacteria (and LPS), thermophilic Micropolyspora, enzymes and chemicals (Table 2), many of which are known to cause human disease; it is apparent that they may do so partly through their ability to activate nonimmune lymphocytes in a polyclonal fashion. Moreover, it is further possible that patients predisposed to inflammatory lung disease may be unable to regulate responses to local mitogenic stimulation in a proper fashion.

The association between α_1-antiprotease deficiencies and a propensity to inflammatory lung disease (including fibrosing alveolitis) is well known. It is particularly interesting, then, to note that α_1-antiprotease has been shown to inhibit lymphocyte activation by T-cell mitogens (*58,59*). We have extended these observations by showing that α_1-antiprotease inhibits IL-1 activity, including both its T-cell activating properties as well as its ability to stimulate fibroblast proliferation (*60,61*). In addition, certain proteases (including trypsin), which are inhibited by α_1-antiprotease, are known to be B-cell mitogens (*35*). Thus, it seems highly plausible that α_1-antiprotease may not only act by inhibiting proteolytic degradation of lung tissue at the site of an established inflammatory reaction, as has been proposed (*62*), but may also regulate the very initiation of such inflammation as well.

Experimental Animal Models of Immunologic Injury to Lung Parenchyma

Arthus-Type Injury

Experimentally induced, immunologically mediated lung injury was first observed nearly a century ago when it was demonstrated that immunized guinea pigs challenged with antigen administered via the airways went into anaphylactic shock (*63*). The first Arthus-type pulmonary lesions were reported by Opie (*64*), who stated that injection of horse serum through the thoracic wall of an immune rabbit caused local consolidation with a central zone of necrosis. Subsequently, a number of investigators have employed such direct active Arthus models, or in some cases, a reverse passive Arthus approach, in which animals are first injected with antigen intravenously, then challenged with intratracheal injections of antibody, resulting in acute, neutrophil enriched intra-alveolar hemorrhages (*65*).

Circulating Immune Complex Injury

Another experimental approach to studying experimental immunologic lung disease has employed a serum sickness model, characterized by sequestration of circulating immune complexes within the lung. Vaubel (*66*) first described hyalin capillary "thrombi" in lungs of rabbits dying in anaphylactic shock following intravenous injections of serum protein antigen, while Gregory and Rich described similar lesions in rabbits undergoing chronic serum sickness (*67*). Subsequently, Dixon (*68*) and McKinnon et al. (*69*) demonstrated that such lesions in fact represented intravascular precipitates of antigen and antibody. Recently, Brenjens et al. (*70*) extended the chronic serum sickness model to show that, in rabbits making hyperactive antibody responses to daily injections of bovine serum albumin (BSA), immune complexes would deposit along the alveolar capillary walls and interstitium, accompanied by interstitial inflammation and basement membrane thickening. This finding was particularly interesting, since it is well known that patients with immune complex-type diseases, such as rheumatoid arthritis and systemic lupus erythematosus, have an increased likelihood of developing interstitial pulmonary fibrosis (*13*). As mentioned previously, circulating immune complexes have also been demonstrated in patients with "idiopathic" interstitial pulmonary inflammation, in the absence of other manifestations of a connective tissue disorder (*13*).

Autoimmune Injury

Experimental studies using animals models have also clarified mechanisms of lung injury initiated by anti-lung antibody. First, it was demonstrated that the lung shares antigenic determinants with other highly vascular structures such as the renal glomerulus, placenta, liver and spleen (*71*). Antisera against such organs cross-react extensively, although some lung-specific antibody can be demonstrated. A particularly elegant demonstration of such specificity was provided by Pressman (*72*), who developed a paired-isotope method for quantitating small amounts of antibody localization *in vivo*. In this technique, antibody-containing immunoglobulin is radiolabeled with ^{125}I, while a control (nonantibody-containing) immunoglobulin preparation is labeled with ^{131}I, and mixtures of the two labeled preparations injected intravenously. Although all organs will contain both ^{131}I as well as ^{125}I, bound to immunoglobulin molecules present in the vascular compartment and within the interstitial spaces, tissues containing specifically bound antibody will have a $^{125}I/^{131}I$ ratio somewhat greater than that of the blood; it is then possible, by means of simple calculations to determine net increase in ^{125}I present and, hence, the net amount of antibody fixed.

The ability of heterologous anti-lung antibody to cause lung injury has been convincingly demonstrated in several laboratories where, in every case, such injury was characterized by intraalveolar hemorrhage, together with acute glomerulonephritis and proteinuria (*73,74*). Whereas most studies employed antisera prepared against whole lung homogenate, Willoughby and Dixon (*74*) prepared rabbit antibody against partially purified rat pulmonary vascular basement membrane antigen. After isolating the antibody-containing IgG fraction by column chromatography, they radiolabeled it with ^{125}I, and injected it (together with ^{131}I-labeled normal IgG) intravenously into rats. Thus, by using the

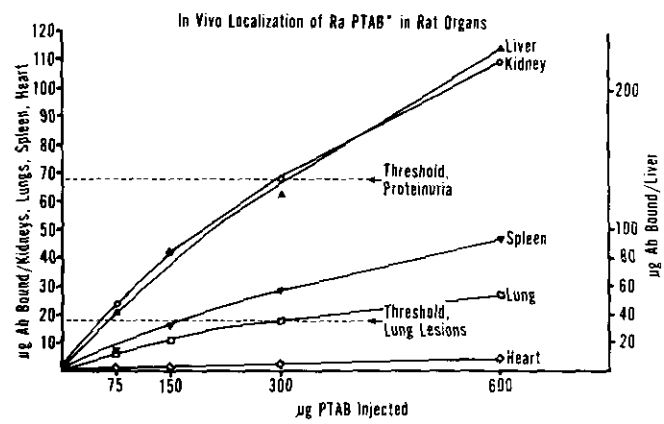


FIGURE 3. Paired-isotope measurements of rabbit anti-rat lung antibody (Ra PTAB) binding *in vivo* following intravenous injection into Sprague-Dawley rats. Each point represents the average value obtained with six rats. Threshold values (broken horizontal lines) give minimal injected dose/organ fixation required for pulmonary hemorrhage and protein-uria From Willoughby and Dixon (*74*) with permission.

paired-isotope technique, they were able to observe the dose-response relationships between antibody binding in the lung and kidney, and the development of pulmonary hemorrhage and glomerulonephritis, respectively (Fig. 3). Simultaneously, immunohistochemical staining demonstrated the anatomic sites of antibody binding (alveolar capillary membranes, glomerular basement membranes and liver and spleen reticulum). Surprisingly, only relatively small amounts of antibody bound to the alveolar capillaries in these animals, doing so in a very focal fashion; instead, most of the antibody bound to the glomerular basement membrane and hepatic and splenic reticulum (*74*). Ultrastructural studies have suggested that the alveolar capillary basement membrane is shielded by a continuous layer of endothelium (in contrast to the glomerular basement membrane, hepatic and splenic reticulum, where basement membranelike antigens come into direct contact with the circulation through "gaps" in the endothelial cell layer) (*75*). Thus, Willoughby and Dixon suggested that perhaps it was necessary for a localized insult or lesion to occur before circulating antibody could bind to alveolar basement membrane antigens (*74*). Recently, Jennings et al. (*76*) have provided direct experimental confirmation of this concept by showing that oxygen-induced injury to alveolar capillary endothelium greatly enhanced binding of circulating anti-basement membrane antibody, and thereby increased the severity of pulmonary injury. All of these animal models closely resemble Goodpasture's syndrome in man, in which pulmonary hemorrhage and glomerulonephritis occur in association with circulating autoantibodies to basement membrane (*77*).

At present, it is not known why autoantibodies to alveolar capillary basement membrane are produced nor, for that matter, what events might trigger acute hemorrhagic episodes in patients with Goodpasture's syndrome. In some cases, there is a history of exposure to hydrocarbon solvents (*78*). In this regard, the sera of normal animals have been demonstrated to contain antigenic fragments of alveolar capillary basement membrane which appear immunologically indistinguishable from those excreted into the urine (*74*). When such urinary antigens are emulsified in Freund's adjuvant and injected back into the host, autoantibody formation occurs (*79*). Thus, it is possible that inhaled environmental agents might increase release of antigenic fragments into the circulation, alter them chemically, or both, thereby prompting an autoimmune response by the host. Such an agent might also cause focal increases in alveolar capillary permeability, thereby facilitating binding of circulating antibody to the capillary basement membrane. On the other hand, recent experimental studies by Izui, Moller, and Coutinho (*80*) have provided provocative evidence indicating that autoantibody production may be the result of polyclonal activation induced by mitogens acting nonspecifically on T-and/or B-cells.

Evidence for cell-mediated immune injury to lung tissue has been most clearly demonstrated in studies of pulmonary allograft rejection. In one such study, Lee et al. (*81*) performed heterotopic heart/lung allografts using inbred rat strains, thereby permitting the use of isograft controls. These investigators demonstrated that early cell-mediated injury to lung allografts, seen at 2 days post-transplant, consisted of perivascular mononuclear infiltrates affecting small and medium-sized pulmonary arteries. Later changes, observed 2 weeks post-transplant, consisted of occlusive endothelial proliferation, perivascular fibrosis and diffuse mononuclear infiltrates throughout the pulmonary parenchyma.

Immunologic Injury Induced by Inhaled Substances

Whereas the preceding models of pulmonary injury caused by circulating immune complexes or autoimmune reactions constitute mechanisms capable of affecting most organs, the unique anatomic relationships of the lung render it vulnerable, in theory, to yet another

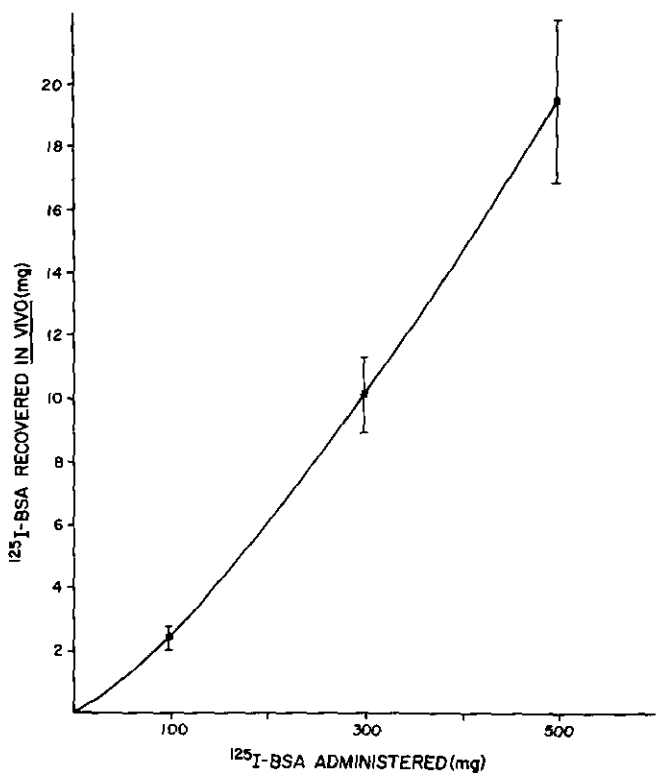


FIGURE 4. Total body uptake of inhaled ^{125}I-labeled bovine serum albumin (BSA), expressed as a function of total ^{125}I-BSA aerosolized. ^{125}I-BSA was administered daily (*100 mg/day*) for 1, 3 or 5 consecutive days to 11, 11, and 10 rabbits, respectively. Rabbits were sacrificed 24 hr after the final dose. Total uptake values were calculated individually by summing radioactivity measurements of organs, plasma, and cumulative urinary excretions, then averaged for each group. Results are expressed as mg equivalents of ^{125}I-BSA recovered. Vertical bars represent standard errors of the mean. From Willoughby and Willoughby (*82*).

mechanism of parenchymal injury; namely, host immune reactions directed against inhaled substances. As noted previously, such a hypothesis has been proposed to describe the pathogenesis of allergic alveolitis. Consequently, there have been numerous attempts to develop an appropriate animal model for such injury in an effort to identify possible pathogenic mechanisms. Obviously, an ideal model would employ well-characterized, immunologically defined agents, administered in aerosol form, and would be characterized by the production of a granulomatous interstitial pneumonitis (*7*). In practice, most models employed to date represent compromises of one or more of these goals. Thus, a variety of antigens, including bovine serum albumin (BSA) (*82*), azobenzenearsonate (*83*), ovalbumin (*84*), PPD (*85*), *M. faeni* (*86,87*), pigeon droppings (*88*), and heterologous serum (*89*) have been used. Antigen challenge has been administered by aerosol inhalation (*82,84,90*), direct intrapulmonary instillation (*65*), or even transtracheal injection (*86*), resulting in injury patterns ranging from pulmonary consolidation (*63*) to a neutrophil-rich intraalveolar hemorrhage (*65*), to no injury at all (*82*).

No general agreement has been reached concerning the mechanism(s) involved. Fried (*63*) originally suggested that injury produced by intratracheal injections of horse serum into immunized rabbits resembled the cutaneous Arthus reaction. Hensley and Fink (*89*) obtained support for this concept by demonstrating antigen, host immunoglobulin and complement (C3) by immunofluorescence staining in lung tissues from monkeys sensitized passively with antibody, then challenged with antigen aerosol; similar findings were observed by Santives et al. (*91*) in the guinea pig. By prior decomplementation with cobra venom factor, Roska et al. (*92*) were able to prevent injury in ovalbumin-immunized guinea pigs challenged with ovalbumin aerosols. Johnson and Ward (*65*) also demonstrated a requirement for complement and neutrophils in the reverse passive Arthus model, and further suggested a role for oxygen-derived free radicals in the production of such injury (*49*).

In contrast, other investigators have failed to detect evidence of immune complex injury in lungs of immunized rabbits challenged with *M. faeni* (*93*) or BSA (*82*). Tuft et al. (*94*) were unable to prevent injury induced by BSA aerosols in sensitized rabbits by prior de-complementation, while Moore et al. (*88*) actually observed an inverse relationship between complement consumption and injury following challenge with a pigeon dropping extract. In one study, Santives et al (*91*) reported that IgE antibody serves to trigger immune complex disease; however, most studies to date have failed to demonstrate a role for homocytotropic antibody in these models (*87,88,92*). In retrospect, convincing models of inhalation-induced, immune complex-type lung injury have been demonstrated primarily in conjunction with selected "enhancing" measures, including simultaneous mitogen inhalation (*57,90*), intramuscular

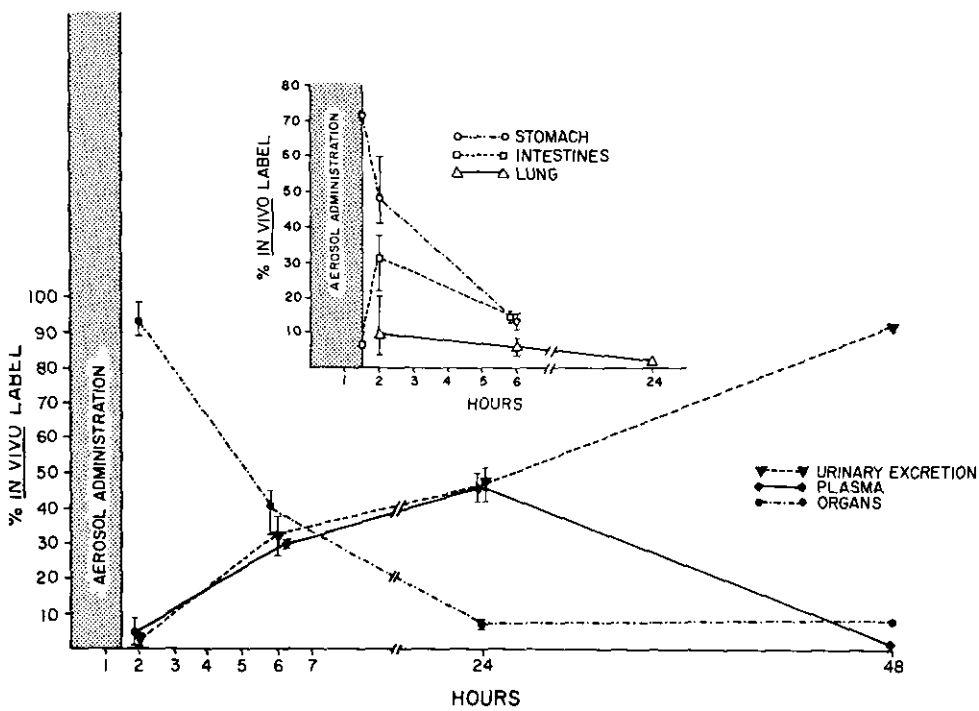


FIGURE 5. Distribution of protein label as a function of time following a single aerosol administration of ^{125}I-BSA. Values are expressed as mean percentage of total *in vivo* label recovered in organs, plasma and cumulative urine in individual rabbits. Organ values were calculated by the paired isotope technique. The insert gives values for three individual organs. Vertical bars give the range of experimental values observed. From Willoughby and Willoughby (*82*).

injections of complete Freund's adjuvant (*91*), or intravascular injections of killed BCG organisms (*88*). The latter two approaches, while effective, may be of questionable relevance to human disease; the potential role of inhaled mitogens, on the other hand, may be particularly relevant.

The ability of humoral antibody or sensitized lymphocytes to passively sensitize test animals to subsequent injury by aerosol challenge has been studied in several laboratories. Adoptive sensitization using serum antibody has been achieved successfully in some instances (*89,95*), but not others (*94,96*). Transfer of sensitized cells has been reported to render the guinea pig susceptible to inhalation injury (*97*); Wilkie et al. (*87*) found that either cells or serum worked satisfactorily. Bice et al. (*98*) were able to adoptively sensitize rabbits to subsequent aerosol challenge by giving intraperitoneal injections of allogeneic lymphoid cells (but not serum) obtained from immunized donors. Other cell-transfer techniques, carried out in outbred animals, have experienced technical difficulties due apparently to histocompatibility differences between donor and recipient or to rapid antibody production by the transferred cells (*89*).

Willoughby and co-workers, in developing an animal model of environmental lung disease, have employed an aerosol challenge system in which antigens and mitogens can be administered to (and inhaled by) rabbits under precisely defined conditions (*82*). The uptake, organ distribution and fate of these proteins *in vivo* has been quantitatively determined by radiolabeling procedures, while their anatomic localization within the lung has been observed directly by labeling them with fluorescein. Under the standardized conditions employed, one can estimate the total body uptake of inhaled protein as a function of the amount nebulized (Fig. 4). Using a modification of the paired isotope system to correct for plasma label, they have measured the organ sequestration of inhaled protein as a function of time following aerosol administration (Fig. 5). While at least 10% of the inhaled protein initially reaches the lung, most is eventually cleared into the gastrointestinal tract and digested. The degraded peptides (which no longer react with anti-BSA antibody) are absorbed into

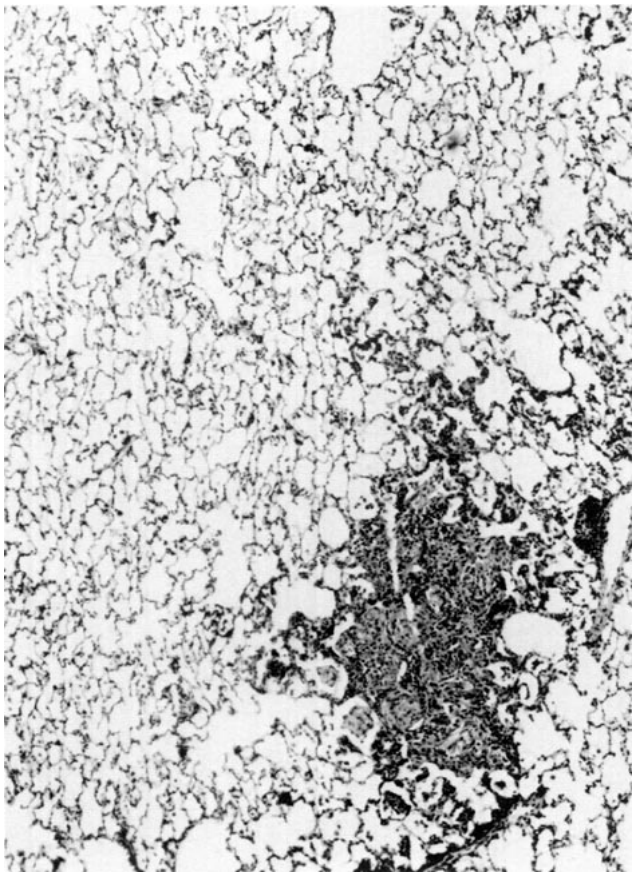


FIGURE 6. Pulmonary histopathology in a BSA-immunized rabbit challenged with BSA aerosol. The small, solitary focus of granulomatous, interstitial inflammation, seen in the lower left, is characteristic of the widely scattered, almost trivial lesions occasionally present in rabbits immunized with BSA in alum depots, and challenged for three consecutive days with BSA aerosols, 100 mg BSA/day (estimated total pulmonary uptake is 480 μg BSA). H&E; ×60.

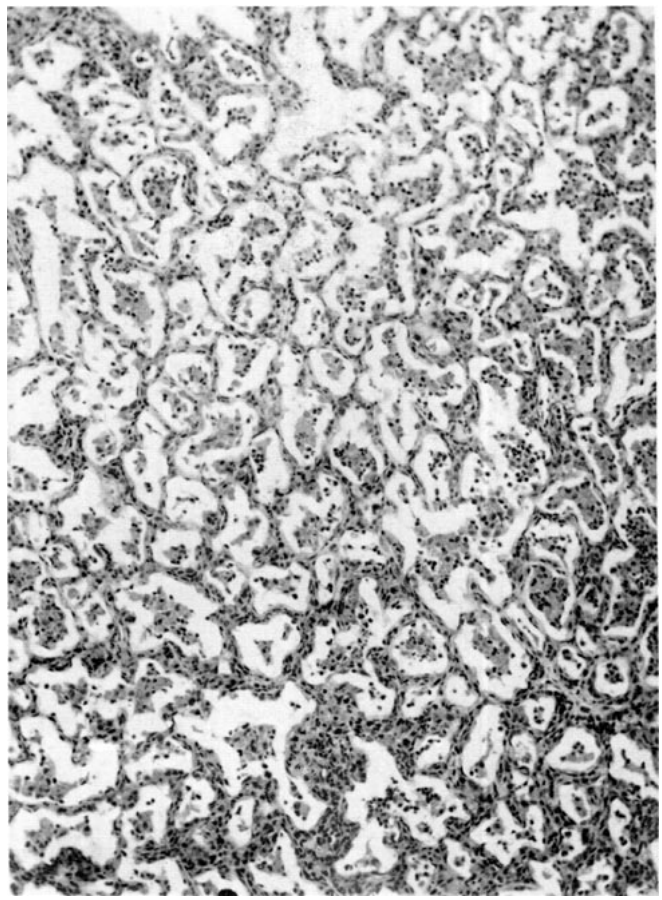


FIGURE 7. Interstitial pulmonary inflammation induced by three daily administrations of Con A aerosol (estimated total pulmonary uptake is 480 μg ConA). H&E; ×110.

the circulation, where they reach maximal concentrations at 24 hr post-aerosol (*82*). By 48 hr post-aerosol, all protein has been degraded, and the radiolabel excreted into the urine. The presence of host immunity to the inhaled protein does not significantly affect the fate of this protein *in vivo* (*82*). The fate of intrapulmonary antigen is critically dependent on the experimental conditions under which it is introduced, however (*99*). For example, direct instillation of antigen-containing solutions into the lung (*82,100*)—or insufflation of antigen aerosols into extirpated perfused lungs (*101*)—results in release of intact antigen into the circulation, due possibly to alterations in tight junctions of cellular barriers (*102*).

Using this defined aerosol administration system, it was next demonstrated that administration of antigen (BSA) aerosols to immunized rabbits (possessing circulating antibody to BSA following repeated intramuscular injections of BSA in alum) induced only minimal pulmonary inflammation (Fig. 6); no injury was observed in previously unimmunized recipients, even following repeated (biweekly) aerosol challenges which, by themselves, eventually induced high titers of circulating anti-BSA antibody (1 mg/mL) after several weeks (*82*).

In contrast, administration of T-cell mitogens (Con A or PHA) in aerosol form induces a diffuse interstitial pneumonitis characterized by interstitial edema and mixed inflammatory infiltrates in alveolar septa (Fig. 7) (*56,57,90*). Immunohistochemical staining has revealed numerous T-lymphocytes in such infiltrates (*90*), while lavage fluids from Con A-treated animals contained significantly increased numbers of T-lymphocytes over those present in saline controls (*103*), suggesting an amplifying process characterized by T-cell proliferation and/or recruitment into the lung. Mitogen-induced interstitial inflammation is reversible, and the lung lesions resolve completely within several days following cessation of aerosol challenge (*57*).

Whereas antigen or mitogen aerosols, given individually, produce trivial or moderate interstitial inflammation, respectively, inhalation of antigen/T-cell mitogen mixtures by immune animals results in devastating pulmonary inflammation with areas of parenchymal necrosis and even infarction (*57,90,104*) (Fig. 8). Occasionally, these are severe enough to cause death within hours of aerosol administration. These pulmonary lesions are characterized by interstitial edema and inflammatory infiltrates of neutrophils, lymphocytes, macrophages and basophils. Immunofluoresence microscopy has demonstrated inhaled antigen, specific antibody and C3 as granular interstitial deposits (Fig. 9), at or near necrotic foci in the lung; such deposits were not observed in animals challenged with antigen or mitogen alone (*57,82,104*). Repetitive weekly challenges with antigen/ mitogen aerosols produce chronic pulmonary injury characterized by areas of focal scarring and interstitial fibrosis (*57*) (Fig. 10).

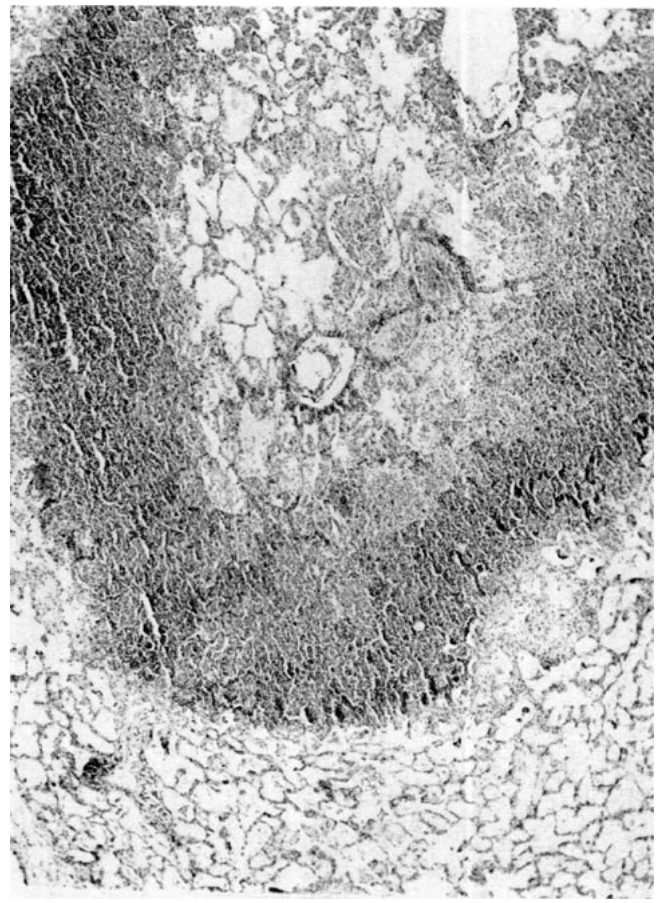


FIGURE 8. Parenchymal necrosis and bland infarction of pulmonary tissue following administration of three consecutive daily aerosols of BSA and Con A to BSA-immunized rabbits. Note the small rim of viable lung tissue on the left showing only interstitial inflammation. (H&E; ×45).

These observations thus demonstrate two pathogenic effects of these phytomitogens. First, they induce interstitial pulmonary inflammation when inhaled, even in an unimmunized host. Second, they trigger formation of pathogenic immune complexes between inhaled antigen, humoral antibody and complement. Since Con A is known to activate T-lymphoctes (and, in some instances, basophils as well) in a polyclonal fashion *in vitro*, we have proposed that its unusual toxicity for the lung results from its ability to activate these cells *in vivo*, again in an amplified, polyclonal fashion (*90*). We further suggest that, whereas under usual circumstances inhaled antigen normally does not come into direct contact with humoral antibody, probably because the type I pneumocytes serve as an effective anatomic barrier (Fig. 11*a*), mediators released by mitogen-stimulated lung lymphocytes and macrophages may alter or disrupt this barrier (Fig. 11*b*), thereby enhancing interactions between intra-alveolar antigen and interstitial/intravascular antibody (Fig. 11*c*). Formation of immune complexes, accompanied by complement

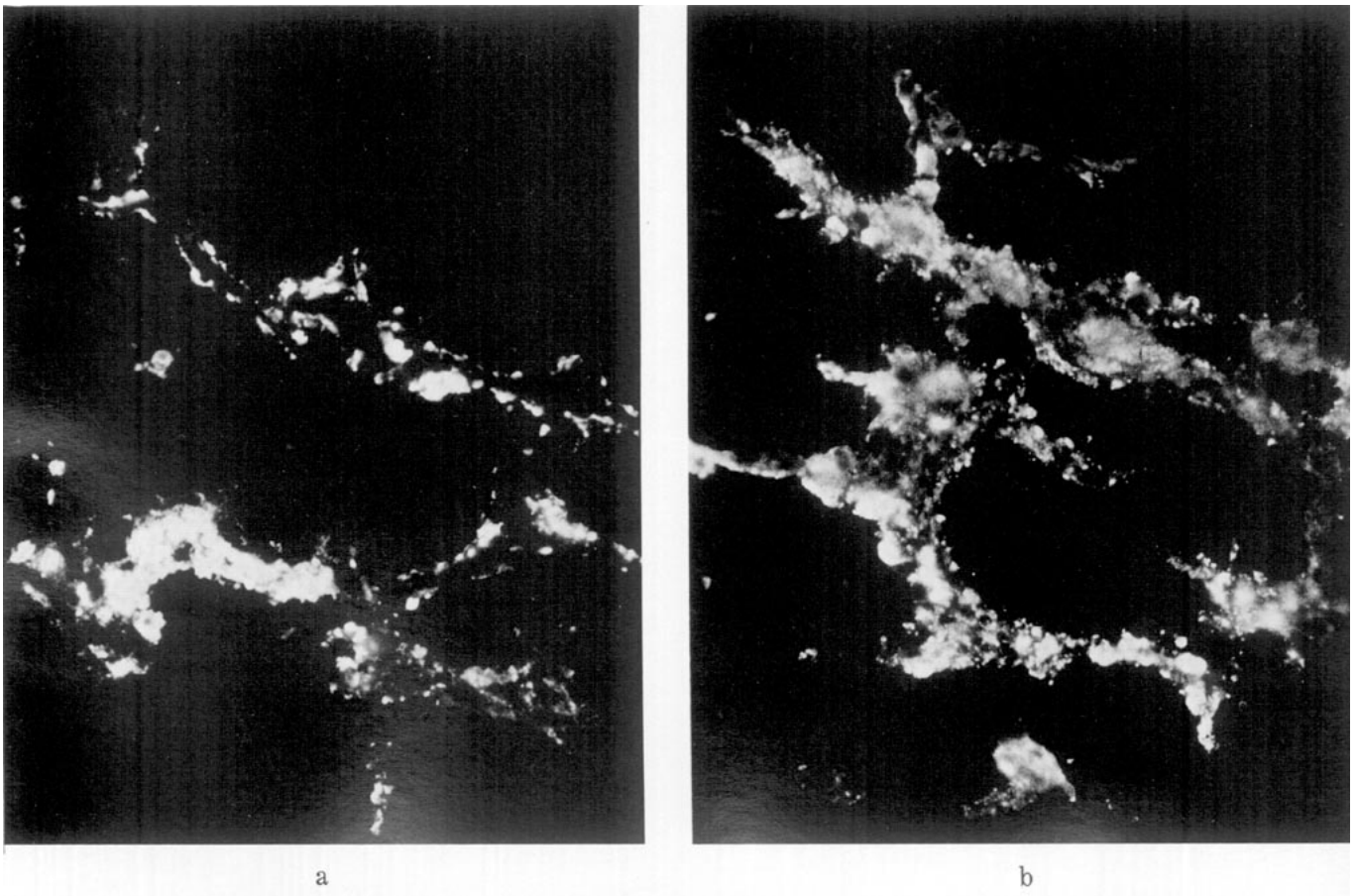


FIGURE 9. Immunofluorescence microscopy. (*a*) Localization of inhaled BSA in the lung of a BSA-immunized rabbit challenged with BSA–Con A aerosol. Note the massive deposits of BSA in markedly thickened septa (lower left) as compared to the delicate deposits along outer surface of septa (top) which are relatively normal in thickness; ×300. (*b*) Demonstration of rabbit IgG in a BSA-immunized rabbit challenged with BSA–Con A aerosol. Note the granular pattern of localization within the thickened septa; ×300. From Willoughby et al. (*57*).

activation, then results in chemotactic attraction of neutrophils into the interstitial spaces (*57*) where, through release of proteolytic enzymes and, possibly, oxygen radicals, the collagen and elastin structural framework is destroyed, resulting in necrosis or dissolution of tissue structure.

To test the hypothesis that inhaled mitogens initiate or "trigger" immune complex formation between inhaled antigen and circulating antibody, Shenker et al. (*104,105*) blocked the responsiveness of lymphocytes to T-cell mitogens *in vivo* by parenteral administration of cholera toxin. Such treatment elevated the cAMP levels of peripheral blood lymphocytes and blocked their proliferative responses to subsequent stimulation by Con A *in vitro*. It also inhibited the tuberculinlike response to intradermal injections of Con A, but did not affect the immune complex-mediated cutaneous Arthus vasculitis (*104,105*), thereby demonstrating a selective inhibition of cell-mediated reactions. If immune complex formation in the lung is dependent upon initiation by mitogen-activated leukocytes, it may be predicted that cholera toxin should inhibit both the mitogen-induced interstitial inflammation and immune complex-mediated parenchymal necrosis. The hypothesis was supported by the experimental data; both forms of pulmonary inflammation were prevented by cholera toxin administration (*104,105*). Not only did these experiments provide critical support for the role of mitogens in "triggering" immune complex formation in the lung, they further suggested that individual susceptibility to environmental lung disease may depend, in part, on the inherent responsiveness of an individual's leukocytes to mitogen activation (*104*).

This conceptual argument was further strengthened by Hollingdale et al. (*104,106*), who showed that decomplementation with purified cobra venom factor (CVF) [which lowered serum complement (CH_{50}) hemolytic titers by 80% and lowered C3 concentrations by 82%] markedly inhibited the cutaneous Arthus vasculitis without affecting the cutaneous inflammatory response to Con A. As expected, CVF also prevented the production of immune complex-mediated parenchymal

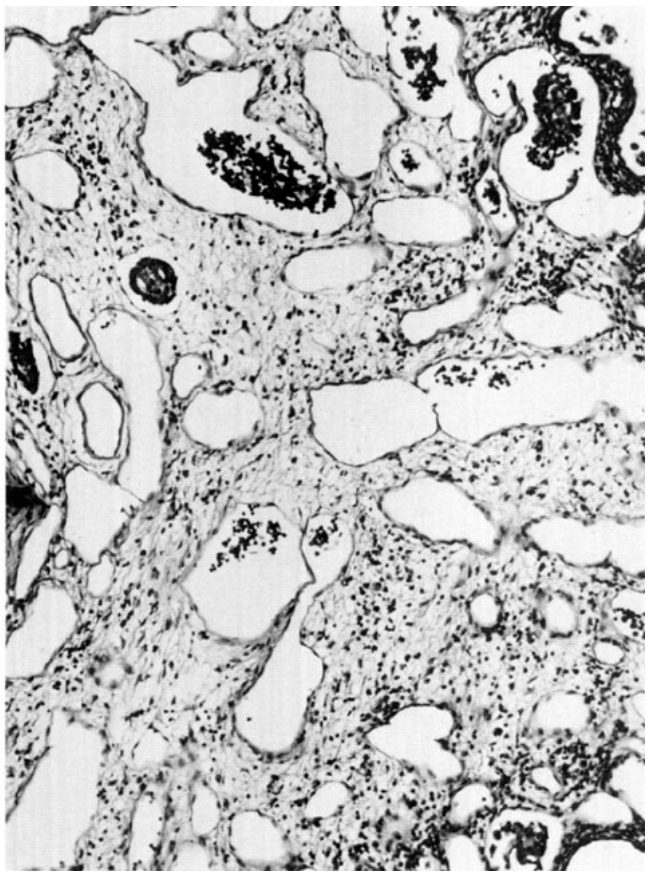


FIGURE 10. Pulmonary fibrosis induced by thrice weekly challenges with BSA–Con A aerosol for 8 weeks. Note scattered round cell infiltrates and distorted residual air spaces. ×110. Willoughby et al. (*57*).

necrosis by antigen/mitogen aerosols, but did not prevent the interstitial inflammation characteristic of injury induced by inhaled mitogen alone.

Recently, we have begun a study of the pathogenic actions of inhaled B-cell mitogens, comparing them with those previously described for T-cell mitogens. Such studies are deemed particularly appropriate, since *M. faeni* has been shown to be a B-cell mitogen (*107*), while another B-cell mitogen, bacterial lipopolysaccharide (LPS), is thought by some to represent the pathogenic agent in cotton dust causing byssinosis (*108*). A variety of types of LPS, derived from different organisms and extracted by either the Westphal or butanol methods, have been found to induce a diffuse interstitial pneumonitis (Fig. 12). Dose-response studies have indicated that inhalation of approximately 100 μg of LPS into the lung for each of three consecutive days results in a severe interstitial inflammation, while inhalation of lesser amounts (10–33 μg) results in little or no inflammation. Moreover, inhalation of LPS also results in a slightly delayed febrile response, generally reaching a maximal value 2–4 hr post aerosol (*103*). Of particular interest, however, is our preliminary finding that LPS does not trigger immune complex injury when administered in conjunction with specific antigen to immunized animals, even though it produces an interstitial pneumonitis fully as severe as that produced by Con A. Thus, initiation of pulmonary immune complex formation by Con A must not represent merely the effects of a nonspecific injury to the alveolar interstitium, but instead must result from some relatively specific property of this lectin in the lung. An obvious possibility is that it may activate intrapulmonary T-cells to release vascular permeability factor (*109*), or mast cells and basophils to release vasoactive amines (*36*), thereby increasing vascular permeability. Alternatively, it may serve as a particularly effective stimulus for local generation of AGEPC or of oxygen-derived free radicals. Regardless of the exact effector system(s) involved, the important point is that, while all polyclonal cell activators which we have tested to date are capable of causing pulmonary interstitial inflammation, some, but not all, also act to trigger local immune complex formation under the appropriate circumstances.

In order to determine whether mitogen-induced lung injury is mediated through release of soluble mediators by mitogen-activated cells, it is first necessary to characterize such mediators *in vitro*, in order to facilitate their subsequent identification in bronchopulmonary lavage fluids. As a first step, we have generated and characterized two rabbit lymphokines, lymphotoxin (LT) and mitogenic factor (MF), together with a monokine, IL-1. [MF appears to be identical to Interleukin 2(IL-2).] These mediators were selected in part because they can be readily measured in a quantitative and reproducible fashion. In addition, MF represents an endogenous mitogen thought to play an important role in the amplification of host immune effector responses (*110*), while IL-1 is generally believed to serve as a central mediator in the acquisition and expression of cellular immune effector systems and, in particular, exerts an important (and, possibly, critical) role in cellular responses to exogenous mitogens (*32*). The physicochemical properties of these rabbit mediators are given in Table 3.

Recently, we have found both IL-1 as well as a LT-like factor in lavage fluid from animals challenged with LPS aerosols (*103*). Thus, we suggest that IL-1 can be released *in vivo* following activation of alveolar macrophages by inhaled mitogen, and that IL-1 so released plays a central role in the production of fever, interstitial inflammation and even the "adjuvant" effects associated with allergic alveolitis in man. Recently, it has been shown that B-cells from such patients are more responsive to stimulation by IL-1 than are cells from control cohorts (*9*).

If polyclonal activation of pulmonary leukocytes by inhaled mitogens is indeed relevant to human environmental lung disease, than at lease some environmental dusts known to cause lung disease should, in turn,

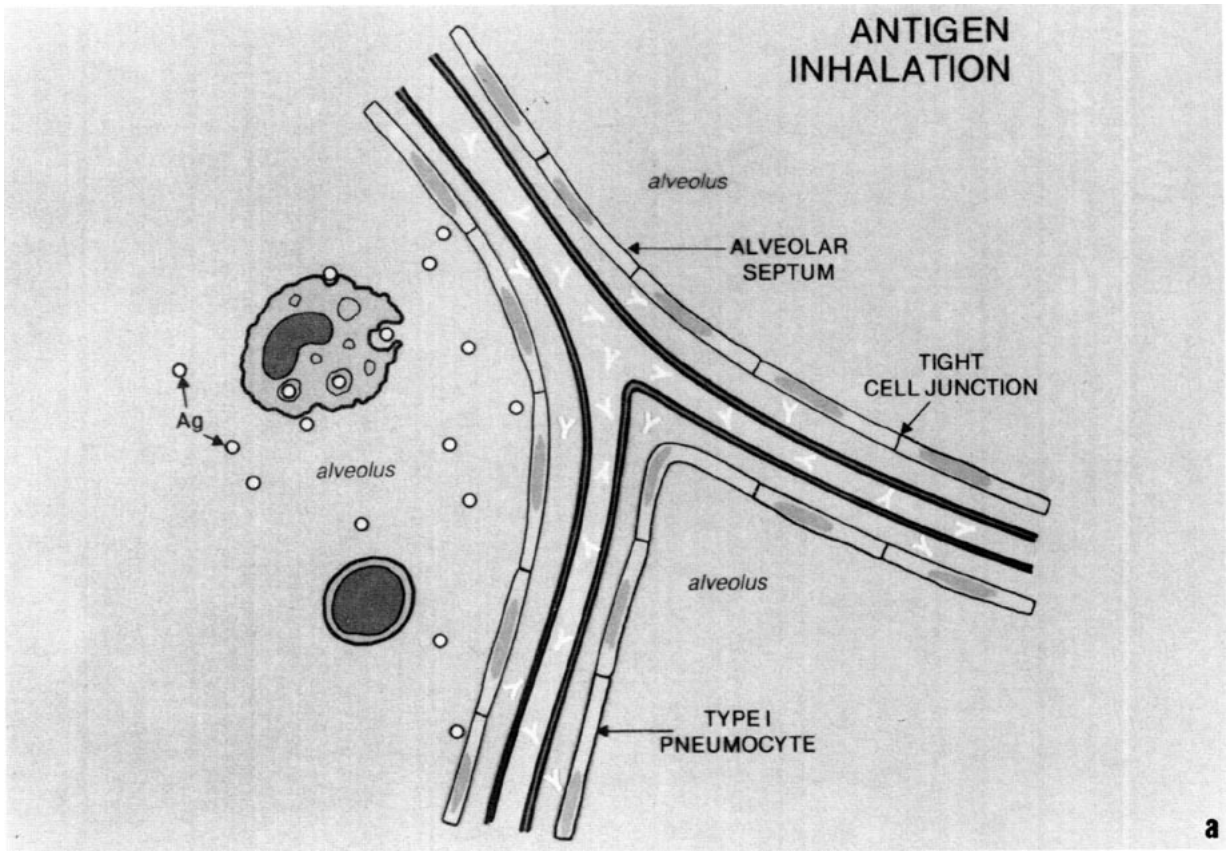
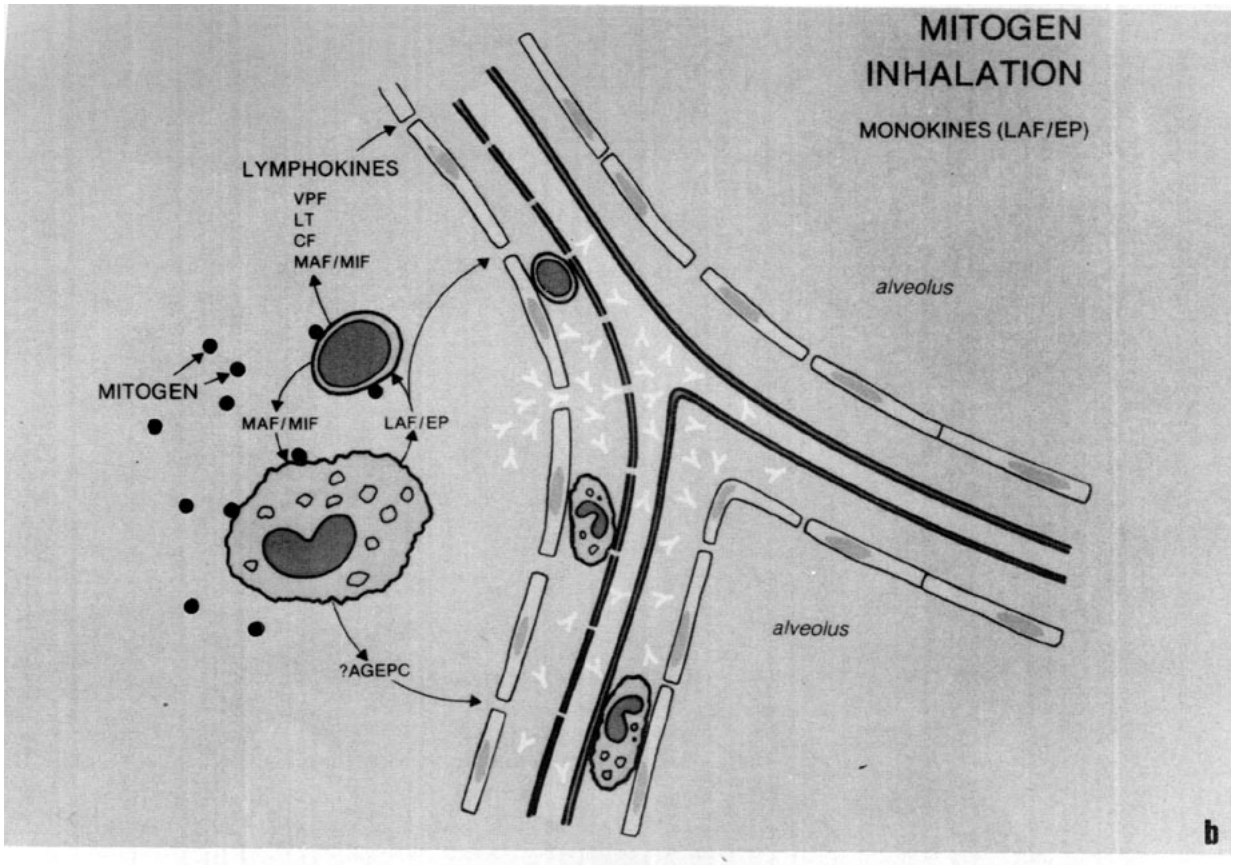


FIGURE 11. A hypothetical model describing parenchymal lung injury by inhaled antigens and mitogens: (*a*) above; (*b*,*c*) facing page. (*a*) Inhalation of antigen alone by immune individuals. Antigen molecules (open circles) within alveolar air sacs do not come into contact with antibody molecules ("Y"-shaped figures) present within the capillaries and interstitial spaces. Although some T-lymphocytes are present in the air spaces, the number which are specifically immune to any particular antigen is negligible, and cell-mediated immune reactions are not initiated. (*b*) In contrast, inhaled mitogens (closed circles) can activate lymphocytes in a polyclonal fashion, thus resulting in a significant local inflammatory response. Direct activation of intrapulmonary lymphocytes and, in some cases, of alveolar macrophages as well, results in local mediator release which further amplifies the local inflammatory process, and may also alter tight cellular junctions between Type I pneumocytes and possibly endothelial cell junctions in addition. Although accessibility of intraalveolar substances to circulating antibody is now greatly enhanced, no immune complexes form in the absence of inhaled antigen. (*c*) When immune individuals inhale mixtures of antigen and mitogen (or when the dust particles possess both antigenic and mitogenic properties), leukocytes are first activated to release mediators which, in turn, promote formation of immune complexes between intraalveolar antigen and circulating antibody. Complement is then activated, leading to neutrophil recruitment, lysosomal enzyme release and oxygen-derived free-radical generation. Necrotizing injury and subsequent fibrosis results from enzymatic destruction of the collagen, basement membrane and elastin framework of the pulmonary interstitium. (Abbreviations: VPF, vascular permeability factor; LT, lymphotoxin; CF, lymphocyte-derived chemotactic factor; MAF, macrophage-activating factor; MIF, macrophage migration inhibitory factor; LAF, lymphocyte activating factor, also known as IL-1; EP, endogenous pyrogen, now thought to be identical to LAF; AGEPC, acetyl glyceryl ether phosphorylcholine, the chemical name for platelet activating factor).

possess polyclonal cell-activating properties. Studies from our laboratory and from Burrell's laboratory (*107*) have shown that the causative agent for farmer's lung, *M. faeni*, is mitogenic for B-lymphocytes from immunologically naive or even germ-free animals (*103*) and that it also stimulates macrophages to release IL-1 (*103*). Moreover, *M. faeni* skin tests are known to be of little value, since they often give "false positive" reactions in nonimmune individuals (*7*) (as do Con A and PHA). Thus, this organism may indeed be acting as a polyclonal activator in man. In a rather different pulmonary disorder, byssinosis, respiratory symptoms are caused by an agent in respirable cotton dust which is thought by some to be LPS (*108*), a known B-cell mitogen and macrophage activator (*35*).

Since there are many different organic and inorganic substances present in the environment which are known to be mitogenic (Table 2), the potential role of polyclonal cell activation as a cause of environmental lung disease deserves serious consideration. Far from simply being "toxic" agents which cause "nonspecific" injury, they activate cells by binding to specific receptors; thus, their apparent lack of specificity, as compared to antigenic stimulation, simply reflects the widespread distri-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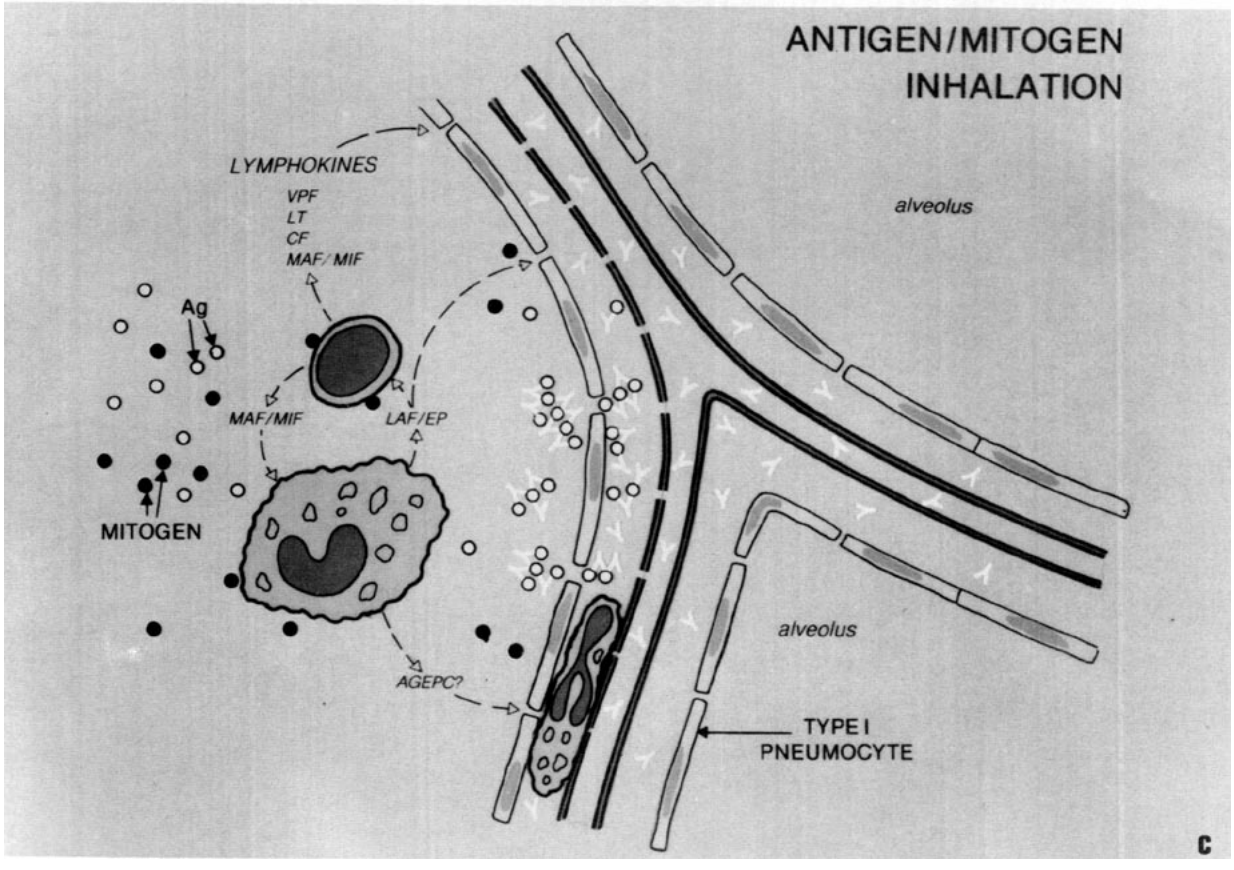
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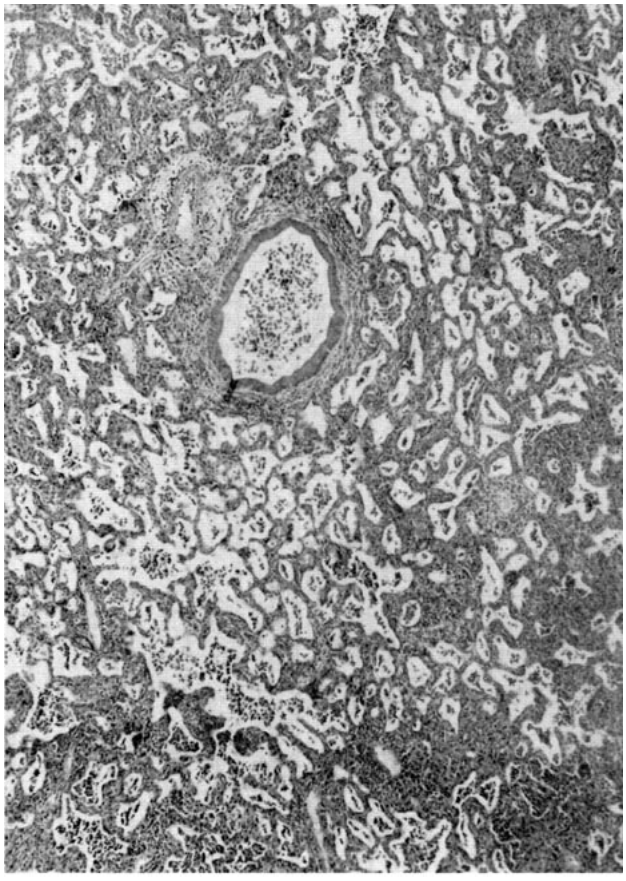

FIGURE 12. Interstitial pulmonary inflammation induced in rabbits by three daily aerosol administrations of LPS aerosol. The estimated total pulmonary uptake is 150 μg LPS. H&E; ×110.

bution of mitogen receptors on cell surfaces (*35*). Moreover, they activate precisely the same inflammatory effector systems as immunologically specific reactions, and thus will closely resemble immunologic injury, except that there will be no obvious relationship between the presence or absence of disease and true host immunity *per se*.

Epilogue

Recent scientific advances in understanding the inflammatory response, both at the biochemical as well as cell-biology level, have presaged comparable advances in our understanding of pulmonary injury. The multitude of effector mechanisms which have been identified and defined to date demonstrate elements of complexity and redundance in the inflammatory response, including highly effective amplification and control systems. To attempt to relate a given disease to the action of a single effector system may well represent an over-simplification. However, it may be entirely feasible to search for, and identify, a single initiating event or process in a disease state. Indeed, many forms

Table 3. Physicochemical parameters of rabbit-derived soluble mediators.

	pI	$S_{20,w}$	$D_{20,w}$	$\bar{v}$	Molecular weight[a]
Lymphotoxin	5.1	3.76	7.37	0.72	44,000
Mitogenic factor	—	2.13	9.63	0.72	19,000
Interleukin-1					
4.6 IL-1	4.6	2.0	12.5	0.73	14,400
7.4 IL-1	7.4	1.8	14.0	0.73	11,600

[a] Molecular weights calculated by the Svedberg equation.

of environmental lung disease can be viewed as the consequence of an inappropriate activation of normal inflammatory effector mechanisms. At the same time, it is well known that attempts to control inflammatory diseases by blocking one or more of the effector systems frequently extract a price of their own, such as increased susceptibility to infection.

Antigen inhalation almost certainly occurs in an ongoing fashion; it probably constitutes a major means for establishing "natural" host immunity, and only infrequently is associated with inflammatory lung disease. Thus, the antigenicity of an inhalant should not imply that it will necessarily cause pulmonary injury, nor, for that matter, should the presence of host immunity to that inhalant. Recognition that inhaled environmental substances possess mitogenic or polyclonal leukocyte-activating properties, on the other hand, should be a cause for concern. If, indeed, the *in vivo* pathogenicity of environmental mitogens parallels their *in vitro* activating properties, as is the case with Con A and LPS, then it should be possible to screen environmental dusts for mitogenic activity as a measure of their disease-causing potential. At the same time, one might be able to also screen inhabitants of that environment for disease susceptibility, by determining the responsiveness of their leukocytes to mitogenic stimulation *in vitro*, using appropriate dust mitogens.

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REFERENCES

1. da Carpi, R.: De Morbus Artificium. Modena, Italy, ca. 1700.
2. Koffler, D., Sandson, J., Carr, R., and Kunkel, H. G. Immunologic studies concerning the pulmonary lesions in Goodpasture's syndrome. *Am. J. Pathol.* 54: 293–301 (1969).
3. Pepys, J. Riddel, R. W., Citron, K. M., and Clayton, Y. M. Precipitins against extracts of hay and fungi in the serum of patients with farmer's lung; aspergillosis, asthma and sarcoidosis. Thorax 17:366–374 (1962).
4. Pepys, J. Hypersensitivity Diseases of the Lungs Due to Fungi and Organic Dusts. S. Karger, New York, 1969.
5. Pepys, J. Immunopathology of allergic lung disease. Clin. Allergy 3:1–22 (1973).

6. Fink, J. N., Barboriak, J. J., and Kaufman, L. Cryptococcal antibodies in pigeon breeder's disease. J. Allergy 41:297–301 (1968).
7. Salvaggio, J. E. Hypersensitivity pneumonitis; state of the art. Chest 75 (Suppl.): 270–274 (1979).
8. Flaherty, D. K., Murray, H. D., and Reed, C. E. Cross-reactions to antigens causing hypersensitivity pneumonitis. J. Allergy Clin. Immunol. 53: 329–335 (1974).
9 Girard, J. P., de Wurstemberger, B., and Fernandes, B. Analysis of cell-mediated hypersensitivity, immune complexes and monocyte-released factor(s) in allergic alveolitis and asymptomatic exposed subjects. Clin. Allergy 8: 455–461 (1978).
10. Pepys, J. Allergic alveolitis. In: The Role of Immunological Factors in Infectious Allergic, and Autoimmune Processes (R. F. Beers and E. G. Bassett, Eds.). Raven Press, New York, 1976, pp. 355–365.
11. Hunninghake, G. W., Gadek, J. E., Lawley, T. J., and Crystal, R. G. Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. J. Clin. Invest. 68: 259–269 (1981).
12. Howell, S. B., and Epstein, W. V. Circulating immunoglobulin complexes in Wegener's granulomatosis. Am. J. Med. 60: 259–268 (1976).
13. Dreisin, R. B., Schwartz, M. I., Theofilopoulos, A. N., and Stanford, R. E. Circulating immune complexes in the idiopathic interstitial pneumonias. N. Engl. J. Med. 298: 353–357 (1978).
14. Jacob, H. S., Craddok, P. R., Hammerschmidt, D. E., and Moldow, C. F. Complement induced granulocyte aggregation. N. Engl. J. Med. 302: 789–794 (1980).
15. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y., and Shearer, G. M. Modulation of inflammation and immunity by cyclic AMP. Science 184: 19–28 (1973).
16. Ansfield, M. J., Kaltreider, H. B., Benson, B. J., and Caldwell, J. L. Immunosuppresive activity of canine pulmonary surface active material. J. Immunol. 122: 1062–1066 (1979).
17. Whaley, K. Biosynthesis of the complement components and the regulatory proteins of the alternative complement pathway by human peripheral blood monocytes. J. Exptl. Med. 151: 501–516 (1980).
18. Pisano, J. J. Chemistry and biology of the kallikrein-kinin system. In: Proteases and Biological Control (E. Reich, D. B. Rifkin and E. Shaw, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, 1975, pp. 199–223.
19. Kaplan, A. P., and Austen, K. F. Activation and control mechanisms of Hageman factor-dependent pathways of coagulation, fibrinolysis, and kinin generation and their contribution to the inflammatory response. J. Allergy Clin. Immunol. 56: 491–506 (1975).
20. Reynolds, H. Y., Fulmer, J. D., Kazmierowski, J. A., Roberts, W. C., Frank, M. M., and Crystal, R. G. Analysis of cellular and protein content of bronch-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. J. Clin. Invest. 59: 165–175 (1977).
21. Mayer, M. M. Complement, Past and Present. The Harvey Lectures, Series 72, Academic Press, New York, 1978, pp. 139–193.
22. Muller-Eberhard, H. J. Complement. Ann. Rev. Biochem., 44: 697–723 (1975).
23. Donaldson, V. H., and Evans, R. R. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C'1 esterase. Am. J. Med. 35: 37–44 (1963).
24. Peters, D. K. Complement and membranoproliferative glomerulonephritis. In: Progress in Glomerulonephritis (P. Kincaid-Smith, A. J. F. d'Apice and R. C. Atkins, Eds.), John Wiley & Sons, New York, 1979, pp. 77–87.
25. Fearon, D. T. Activation of the alternative complement pathway. CRC Crit. Revs. Immunol. 1: 1–32 (1979).
26. Willoughby, W. F., Ford, R. J., and Shin, H. S. Complement activation by lysozyme-DNA complexes. J. Immunol. 111: 296–297 (1973).
27. Rosenstreich, D. L. and Wahl, S. M. Cellular sources of lymphokines. In: Biology of the Lymphokines (S. Cohen, E. Pick and J. J. Oppenheim, Eds.), Academic Press, New York, 1979, pp. 220–225.
28. McCluskey, R. T., Benacerraf, B., and McCluskey, J. W.: Studies on the specificity of the cellular infiltrate in delayed hypersensitivity reactions. J. Immunol. 90: 466–477 (1963).
29. Cohen, S., McCluskey, R. T., and Benacerraf, B. J. Studies on the specificity of the cellular infiltrate of delayed hypersensitivity reactions. J. Immunol. 98: 269–273 (1967).
30. Gery, I., Gershon, R. K., and Waksman, B. H. Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. J. Exptl. Med. 136: 128–142 (1972).
31. Murphy, P. A., Simon, P. L., and Willoughby, W. F. Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte activating factors made by rabbit alveolar macrophages. J. Immunol. 124: 2498–2501 (1980).
32. Simon, P.L., and Willoughby, W. F. The role of subcellular factors in pulmonary immune function: Physicochemical characterization of two distinct species of lymphocyte activating factor produced by rabbit alveolar macrophages. J. Immunol. 126: 1534–1541 (1981).
33. Schmidt, J. A., Mizel, S. B., and Green, I. A fibroblast proliferation factor isolated from human MLR supernatants has physical and functional properties similar to human interleukin 1 (Il-1). Fed. Proc. 40: 1084 (1981).
34. Mizel, S. B., Dayer, J., Krane, S. M., and Mergenhagen, S. E. Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin 1). Proc. Natl. Acad. Sci. (U.S.) 78: 2474–2477 (1981).
35. Oppenheim, J. J., and Rosenstreich, D. L. Signals regulating in vitro activation of lymphocytes. In: Progress in Allergy, Vol. 20 (P. Kallos, B. H. Waksman and A. de Weck, Eds.), S. Kargel, Basel, 1976, pp. 65–194.
36. Siraganian, R. P. Basophil activation by concanavalin A. In: Mitogens in Immunobiology (J. J. Oppenheim and D. L. Rosenstreich, Eds.) Academic Press, New York, 1976, pp. 69–84.
37. Demopoulos, C. A., Pinckard, R. N., and Hanahan, D. J. Platelet activating factor. Evidence for 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). J. Biol. Chem. 254: 9355–9358 (1979).
38. Arnoux, B., Duval, D., and Benveniste, J. Release of platelet-activating factor (PAF-acether) from alveolar macrophages by the calcium ionophore A 23187 and phagocytosis. Eur. J. Clin. Invest. 10: 437–441 (1980).
39. McManus, L. M., Hanahan, D. J., Demopoulos, C. A., and Pinckard, R. N. Pathobiology of the intravenous infusion of acetyl glyceryl ether phosphorylcholine (AGEPC), a synthetic platelet-activating factor (PAF), in the rabbit. J. Immunol. 124: 2919–2924 (1980).
40. Benveniste, J. Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils. Nature 249: 581–582 (1974).
41. Vargaftig, B. B., Lefort, J., Chignard, M., and Benveniste, J. Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. Eur. J. Pharmacol. 65: 185–192 (1980).
42. Kuehl, F. A., and Egan, R. W. Prostaglandins, arachidonic acid, and inflammation. Science 210: 978–984 (1980).
43. Arnoux, B. Durand, J. Rigaud, M., Masse, R., and Benveniste, B. Arachidonic acid metabolites in rat alveolar macrophages. Am. Rev. Respir. Dis. (Suppl.) 123: 50 (1981).
44. Hyman, A. L., Spannhake, E. W., and Kadowitz, P. J. Prostaglandins and the lung. Am. Rev. Respir. Dis. 117: 111–136 (1978).
45. Lewis, R. A., Drazen, J. M., Figueiredo, J. C., Corey, E. J., and Austen, K. I. A review of recent contributions of biologically active products of arachidonate conversion. Int. J. Immunopharmacol. 4: 85–90 (1982).
46. Stenson, W. F., and Parker, C. W. Prostaglandins, macrophages, and immunity. J. Immunol. 125: 1–5 (1980).
47. Nathan, C. F., Silverstein, S. C., Brukner, L. H., and Cohn, Z.

A. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exptl. Med. 149: 100–113 (1979).
48. McCord, J. M., and Wong, K. Phagocyte-produced free radicals: role in cytotoxicity and inflammation. In: Oxygen, Free-Radicals and Tissue Damage (Ciba Symposium 65), Amsterdam, 1979, pp. 343–351.
49. McCormick, J. R., Harkin, M. M., Johnson, K. J., and Ward, P. A. Suppression by superoxide dismutase of immune-complex-induced pulmonary alveolitis and dermal inflammation. Am. J. Pathol. 102: 55–61 (1981).
50. Sacks, T. Moldow, C. F., Craddock, P. R., Bowers, T. K., and Jacob, H. S. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. J. Clin. Invest. 61: 1161–1167 (1978).
51. Webster, R. O., Larsen, G. L., and Henson, P. M. Lack of inflammatory effects on the rabbit lung of intravascular complement activation. Fed. Proc. 40: 767 (1981).
52. Ebert, R. H., and Grant, L. The experimental approach to the study of inflammation. In: The Inflammatory Process (B. W. Zweifach, L. Grant and R. T. McCluskey, Eds.), Academic Press, New York, 1974, pp. 4–49.
53. Schatz, M. Patterson, R., and Fink, J. Immunologic lung disease. N. Engl. J. Med. 300: 1310–1320 (1979).
54. Wilson, C. B., and Dixon, F. J. Immunologic mechanisms in nephritogenesis. Hosp. Prac. 14: 57–69 (1979).
55. Oppenheim, J. J., and Rosenstreich, D. L. (Eds.) Mitogens in Immunobiology. Academic Press, New York, 1976.
56. Willoughby, W. F., and Barbaras, J. E. A comparative study of pulmonary injury following antigen and mitogen inhalation. Lab Invest. 32: 460–461 (1975).
57. Willoughby, W. F., Willoughby, J.B., Cantrell, B. B., and Wheelis, R. In vivo responses to inhaled proteins. II. Induction of interstitial pneumonitis and enhancement of immune complex-mediated alveolitis by inhaled Con A. Lab. Invest. 40: 399–414 (1979).
58. Bata, J., Deviller, P., and Colobert, L. Inhibition de la biosynthèse du DNA chez les lymphocytes humains par l'effet de l'alpha-1 antitrypsine. Compt. Rend Acad. Sci. (Paris) 285: 1499–1501 (1977).
59. Breit, S. N., Luckhurst, E., and Penny, R. The effect of α_1 antitrypsin on the proliferation response of human peripheral blood lymphocytes. J. Immunol. 130: 681–686 (1983).
60. Simon, P. L., Willoughby, J. B., and Willoughby, W. F. Inhibition of T-cell inactivation by alpha-1 antiprotease is reversed by purified Interleukin-1. In: Interleukins, Lymphokines and Cytokines (J. J. Oppenheim, Ed.), Academic Press, New York, 1983, pp. 487–492.
61. Willoughby, W. F., Willoughby, J. B., and Simon, P. L. Alpha-1 antitrypsin modulates biological activity of Interleukin-1. Immunobiology 163: 298–299 (1982).
62. Ingram, R. H., Jr. Chronic bronchitis, emphysema, and chronic airway obstruction. In: Principles of Internal Medicine (K. J. Isselbacher, R. D. Adams, E. Braunwald, et al. Eds.), McGraw-Hill, New York, 1980, pp. 1235–1247.
63. Fried, B. M. Allergic lobar pneumonia. J. Exptl. Med. 57: 111–119 (1933).
64. Opie, E. Inflammatory reaction of the immune animal to antigen (Arthus phenomenon) and its relation to antibodies. J. Immunol. 9: 231–245 (1924).
65. Johnson, K. J., and Ward, P. A. Acute immunologic pulmonary alveolitis. J. Clin. Invest. 54: 349–357 (1974).
66. Vaubel. E. Die Eiweissuberempfindlichkeit des Bindegewebes. Beitr. Pathol. Anat. Ziegler 89: 374–418 (1932).
67. Gregory, J. E., and Rich, A. R. The experimental production of anaphylactic pulmonary lesions with the basic characteristics of rheumatic pneumonitis. Bull. Johns Hopkins Hosp. 78: 1–12 (1946).
68. Dixon, F. J. The use of I^{131} in immunologic investigation. J. Allergy 24: 547–555 (1953).
69. McKinnon, G. E., Andrews, E. C., Heptinstall, R. H., and Germuth, F. G. An immunohistochemical study on the occurrence of intravascular antigen-antibody precipitation and its role in anaphylaxis in the rabbit. Bull. Johns Hopkins Hosp. 101: 258–280 (1957).
70. Brentjens, J. R., O'Connell, D. W., Pawlowski, I. B., Hsu, K. C., and Andres, G. A. Experimental immune complex disease of the lung. The pathogenesis of a laboratory model resembling certain human interstitial lung diseases. J. Exptl. Med. 140: 105–125 (1974).
71. Baxter, J. H., and Goodman, H. C. Nephrotic serum nephritis in rats. I. Distribution and specificity of the antigen responsible for the production of nephrotic antibodies. J. Exptl. Med. 104: 467–485 (1956).
72. Pressman, D., Day, E. D., and Blau, M. The use of paired labeling in the determination of tumor-localizing antibodies. Cancer Res. 17: 845–850 (1957).
73. Hagadorn, J. E., Vazquez, J. J., and Kinney, T. R. Immunopathologic studies of an experimental model resembling Goodpasture's syndrome. Am. J. Pathol. 57: 17–25 (1969).
74. Willoughby, W. F., and Dixon, F. J. Experimental hemorrhagic pneumonitis produced by heterologous anti-lung antibody. J. Immunol. 104: 28–37 (1970).
75. Rhodin, J. A. G. An Atlas of Ultrastructure. W. B. Saunders, Philadelphia, 1963.
76. Jennings, L., Roholt, O. A., Pressman, D., Blau, M., Andres, G. A., and Brentjens, J. R. Experimental anti-alveolar basement membrane antibody-mediated pneumonitis. I. The role of increased permeability of the alveolar capillary wall induced by oxygen. J. Immunol. 127: 129–134 (1981).
77. Martinez, J. S., and Kohler, P. F. Variant "Goodpasture's Syndrome"? The need for immunologic criteria in rapidly progressive glomerulonephritis and hemorrhagic pneumonitis. Ann. Int. Med. 75: 67–76 (1971).
78. Glassock, R. J., and Brenner, B. M. Glomerulopathies associated with multisystem and heredofamilial diseases. In: Principles of Internal Medicine (K. J. Isselbachler, R. D. Adams, E. Braunwald, R. G. Petersdorf, and J. D. Wilson, Eds.) McGraw-Hill, New York, 1980, pp. 1320–1327.
79. Lerner, R. A., and Dixon, F. J. The induction of acute glomerulonephritis in rabbits with soluble antigens isolated from normal homologous and autologous urine. J. Immunol. 100: 1277–1287 (1968).
80. Moller, E., Strom, H., and Al-Balaghi, S. Role of polyclonal activation in specific immune responses. Scand. J. Immunol. 12: 177–182 (1980).
81. Lee, S., Willoughby, W. F., Smallwood, C. J., and Richards, O. W. Heterotopic heart and lung transplantation in the rat. Am. J. Pathol. 59: 279–297 (1970).
82. Willoughby, J. B., and Willoughby, W. F. In vivo responses to inhaled proteins. I. Quantitative analysis of antigen uptake, fate and immunogenicity in a rabbit model system. J. Immunol. 119: 2137–2146 (1977).
83. Richerson, H. B. Acute experimental pneumonitis in the guinea pig. J. Lab. Clin. Med. 79: 745–757 (1972).
84. Richerson, H. B. Seidenfeld, J. J., Ratajczak, H. V., et al. Chronic experimental interstitial pneumonitis in the rabbit. Am. Rev. Respir. Dis. 117: 5–13 (1978).
85. Miyamoto, T., and Kabe, J. The lungs as the site of delayed-type hypersensitivity reactions in the guinea pig. J. Allerg. 47: 181–185 (1971).
86. Salvaggio, J., Phanuphak, P., Stanford, R., Bice, D., and Claman, H. Experimental production of granulomatous pneumonitis. J. Allergy Clin. Immunol. 56: 364–380 (1975).
87. Wilkie, B., Pauli, B., and Gygax, M. Hypersensitivity pneumonitis: experimental production in guinea pigs with antigens of *Micropolyspora faeni*. Pathol. Microbiol. 39: 393–411 (1973).
88. Moore, V. L., Hensley, G. T., and Fink, J. N. An animal model of hypersensitivity pneumonitis in the rabbit. J. Clin. Invest. 56: 937–944 (1975).
89. Hensley, G. T., Fink, J. N., and Barboriak, J. J. Hypersensitivity pneumonitis in the monkey. Arch. Pathol. 97: 33–38 (1974).
90. Willoughby, W. F., Barbaras, J. E., and Wheelis, R. Immunologic mechanisms in experimental interstitial pneumonitis. Chest

69 (Suppl.): 290–294 (1976).
91. Santives, T., Roska, A. K., Hensley, G. T., Moore, V. L., Fink, J. N., and Abramoff, P. Immunologically induced lung disease in guinea pigs. J. Allergy Clin. Immunol. 57: 582–594 (1976).
92. Roska, A. K. B., Garancis, J. C., Moore, V. L., and Abramoff, P. Immune-complex disease in guinea pig lungs. I. Elicitation by aerosol challenge, suppression with cobra venom factor, and passive transfer with serum. Clin. Immunol. Immunopathol. 8: 213–224 (1977).
93. Kawai, T., Salvaggio, J., Lake, W., and Harris, J. O. Experimental production of hypersensitivity pneumonitis with bagasse and thermophilic actinomycete antigen. J. Allergy Clin. Immunol. 50: 276–288 (1972).
94. Tuft, D. S., Giddens, W. E., Fairchild, G. A., and Van Arsdel, P. P. The mechanism of hypersensitivity pneumonitis in the rabbit. J. Allergy Clin. Immunol. 49: 103 (1972).
95. Richerson, H. B. Acute hypersensitivity in the rabbits: an experimental model of allergic alveolitis. J. Lab. Clin Med. 76: 991–992 (1970).
96. Richerson, H. B. Varieties of acute immunologic damage to the rabbit lung. Ann. N.Y. Acad. Sci. 221: 340–360 (1974).
97. Miyamoto, T., Kabe, J., Noda, M., Kobayashi, N., and Miura, K. Physiologic and pathologic respiratory changes in the delayed-type hypersensitivity reaction in guinea pigs. Am. Rev. Respir. Dis. 103: 509–515 (1971).
98. Bice, D. E., Salvaggio, J., and Hoffman, E. Passive transfer of experimental hypersensitivity pneumonitis with lymphoid cells in the rabbit. J. Allergy Clin. Immunol. 58: 250–262 (1976).
99. Willoughby, W. F., and Willoughby, J. B. Antigen handling. In: Immunology of the Lung (J. Bienenstock, Ed.), McGraw-Hill, New York, in press.
100. Bensch, K. G. Dominguez, E., and Liebow, A. A. Absorption of intact protein molecules across the pulmonary air-tissue barrier. Science 157: 1204–1207 (1967).
101. Braley, J. F., Dawson, C. A., Moore, V. L., and Cozzini, B. O. Absorption of inhaled antigen into the circulation of isolated lungs from normal and immunized rabbits. J. Clin. Invest. 61: 1240–1246 (1978).
102. Rhodes, R. F., and Karnovsky, M. J. Loss of macromolecular function associated with surgical trauma to the intestine. Lab. Invest. 25: 220–229 (1971).
103. Willoughby, W. F., and Willoughby, J. B. Unpublished observations.
104. Shenker, B. J. Willoughby, W. F., Hollingdale, M. R., and Mann, T. N. In vivo responses to inhaled proteins. III. Inhibition of experimental immune complex pneumonitis after suppression of peripheral blood lymphocytes. J. Immunol. 124: 1763–1772 (1980).
105. Shenker, B. J., Mann, T. N., and Willoughby, W. F. Role of polyclonal cell activation in the initiation of immune complex-mediated pulmonary injury following antigen inhalation. Environ. Health Perspect. 35: 43–53 (1980).
106. Hollingdale, M. R., and Willoughby, W. F. Participation of serum complement in experimental hypersensitivity pneumonitis. Am. Rev. Respir. Dis. 117: 68 (1978).
107. Smith, S. M., Snyder, I. S., and Burrell, R. Mitogenic response to *Micropolyspora faeni* cell walls. J. Allergy Clin. Immunol. 65: 298–304 (1980).
108. Rylander, R. Bacterial toxins and etiology of byssinosis. Chest 79: 34s–38s (1981).
109. Sobel, A. T., Branellec, A. I., Blanc, C. J., and Lagrue, G. A. Physicochemical characterization of a vascular permeability factor produced by Con A-stimulated human lymphocytes. J. Immunol. 119: 1230–1234 (1977).
110. Gately, C. L., Gately, M. K., and Mayer, M. M. The production of lymphotoxin by lymphoid cells stimulated with purified mitogenic factor. A possible amplification mechanism of cellular immunity and allergy. J. Immunol. 116: 669–675 (1976).